Maternal Thyroid Function and its Effects on Adverse Pregnancy Outcome

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Submitted by

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ABSTRACT

The aims of this thesis are firstly, to establish reference ranges of serum thyroid stimulating hormone (TSH), free triiodothyronine (FT3) and free thryroxin (FT4) at 11-13 weeks' gestation in singleton and twin pregnancies and to examine the effect of maternal characteristics and serum antithyroid antibodies and free ß-hCG on the levels of TSH, FT3 and FT4, and secondly to investigate the possible association between maternal thyroid dysfunction in pregnancies complicated by fetal death, preeclampsia (PE), delivery of small for gestational age (SGA) neonates, preterm delivery and fetal aneuploidies.

The study population was derived from a prospective screening study for adverse obstetric outcomes in 4,852 women attending for their routine first hospital visit in pregnancy at 11^{+0}-13^{+6} weeks' gestation. In some of the pregnancy complication groups, we identified additional cases that were examined after screening period. Serum concentrations of FT3, FT4, TSH, anti-TPO and anti-Tg were measured by immunoassay using direct, chemiluminometric technology.

In normal pregnancy (n=4318), TSH increased whereas FT3 and FT4 decreased with gestation and all three were lower in Afro-Caribbean than in Caucasian women. Serum FT3 and FT4 decreased, but TSH did not change significantly with maternal age, TSH and FT3 increased whereas FT4 decreased with body mass index, TSH decreased whereas FT3 and FT4 increased with serum free ß-hCG. In the antibody positive group, compared to the negative group, median TSH was higher and median FT3 and FT4 were lower.

In 45% of women with known hypothyroidism (n=164) diagnosed before pregnancy and receiving levothyroxine at least one of the three biochemical tests was suggestive of persistent hypothyroidism. In pregnancies resulting in miscarriage or fetal death (n=202), the median serum TSH was increased and FT4 was decreased. In pregnancies that developed PE (n=102), there was evidence of hypothyroidism and increased serum TSH was observed in 5 times as many cases with PE compared with those who did not develop PE. In pregnancies delivering SGA neonates (n=212) and in those ending in spontaneous early preterm delivery (n=102) maternal thyroid function was not significantly different from pregnancies with normal outcome. In pregnancies with fetal trisomy 21 (n=30) free ß-hCG was increased and TSH was decreased and in cases with trisomy 18 (n=25) free ß-hCG was decreased and TSH was increased. In normal twin pregnancies (n=235), compared to singletons, serum FT4 was not significantly different but TSH was about 40% lower. The levels of serum TSH and FT4 were similar in dichorionic and monochorionic twins, with or without twin-to-twin transfusion syndrome (n=19) and there were no significant differences between the three groups in serum free ß-hCG.

The thesis established reference ranges of maternal thyroid function in early pregnancy and demonstrated altered function in association with certain pregnancy complications.

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I am indebted to my parents and sister for always being supporting and loving during good and bad times. This study was funded by the Fetal Medicine Foundation (UK Registered Charity No: 1037116) and the Ministry of Higher Education of the United Arab Emirates. I would also like to thank Sheikh Nahyan Bin Mubarak Al Nahyan for his continuous support.

I carried out extensive literature searches on maternal endocrine changes in physiological and pathological pregnancies to identify the area of research that could be investigated within the setting of Fetal Medicine Centre and selected the topic of the Thesis. I searched the Fetal Data base to identify a period with stored maternal blood samples which I extracted for analysis and secured the necessary funding. I developed a research file in excel containing maternal demographic characteristics, findings from the 11-13 weeks visit and pregnancy outcome. In cases of pregnancy complications, such as preeclampsia, I examined the patient files to cormirm the diagnosis. I collected the results of maternal thyroid function and entered the data into the research file. I carried out the statistical analysis and wrote the scientific publications and this thesis.

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TABLE LEGENDS

ABBREVIATIONS

TBG	Thyroxine-binding globulin
TBPA	Thyroxine-binding prealbumin
TTR	Transthyretin
TG	Thyroglobulin
TSH	Thyroid stimulating hormone
TRH	Hypothalamic TSH releasing hormone
TH	Thyroid hormones
T3	Triiodothyronine
T4	Thyroxine
FT3	Free triiodothyronine
FT4	Free thyroxine
rT3	Inactive metabolite 3, 3', 5'-triiodothyronine
TPO	Thyroperoxidase
Anti-TG	Thyroglobulin antibody
Anti-TPO	Thyroid peroxidase antibody
DIT	Diiodotyrosine
MIT	Monoiodotyrosine
hCG	Human chorionic gonadotropin
H_2O_2	hydrogen peroxide
cAMP	Cyclic adenosine monophosphate
CRL	Fetal crown-rump length
DC	Dichorionic twins
MC	Monochorionic twins
TTTS	Twin-to-twin transfusion syndrome
I^-	Iodide
I2	Iodine
IUD	Intrauterine death
NT	Fetal nuchal translucency thickness
HT	Hashimotos thyroiditis
WHO	World Heath Organisation
ORD	Outer ring deiodination
IRD	Inner ring deiodination
SeC	Selenocysteine
Treg	Regulatory T cells
Th1	T-helper cells type 1
Th2	T-helper cells type 2
AE	Acridium ester label
T2-BGG	Diiodothyronin-bovine gamma globulin complex
D1	Deiodinase 1
D2	Deiodinase 2
D3	Deiodinase 3
pmol/L	Pico moles/Liter
mU/L	Milli-international units/litre
U/ml	International units/milli-litre

Chapter 1 Introduction

ABSTRACT

This chapter is divided into 6 sections. The first section presents an overview of the anatomy, embryology, and physiology of the thyroid gland and the role of thyroid hormones.

The second section describes thyroid function in pregnancy, including physiological changes in the mother, thyroid function in the fetus and transfer of maternal thyroid hormones to the fetus.

The third and fourth sections describe thyroid dysfunction, including clinical hypothyroidism, clinical hyperthyroidism and euthyroid autoimmune thyroiditis, and subclinical hypothyroidism, their associated obstetric complications and the effect of treatment of thyroid dysfunction.

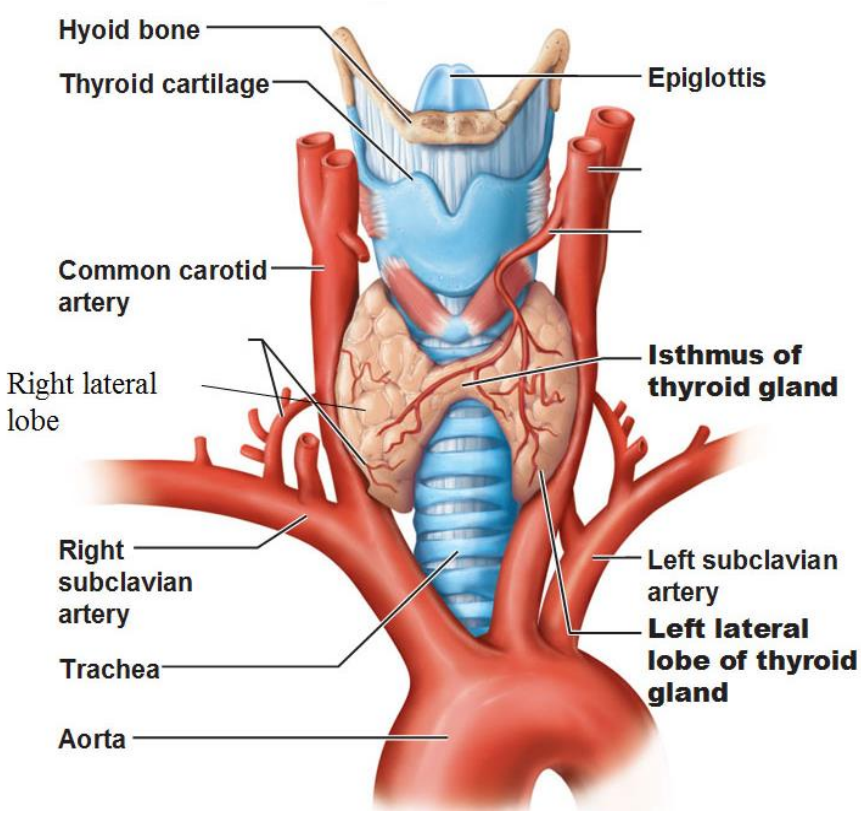
The fifth section describes what is known on maternal thyroid function in normal pregnancies and in those complicated by miscarriage and stillbirth, fetal aneuploidies, preeclampsia, preterm delivery and delivery of small for gestational age neonates

The sixth section summarises the objectives of the studies in this thesis.

1.1 THYROID PHYSIOLOGY AND THE THYROID GLAND

1.1.1. Anatomy of the thyroid gland

The thyroid gland is the shape of a butterfly composed of two cone-like lobes joined by the isthmus. It lies just below the cricoid cartilage and wraps itself around the anterior and lateral aspects of the larynx and trachea (Figure 1.1). The lateral lobes extend laterally between the carotid sheath and the sternomastoid muscle.



http://antranik.org/wp-content/uploads/2011/12/thyroid-gland-left-and-right-lateral-lobes-isthmus-and-aorta-and-trachea-anterior-view.jpg

Figure 1.1. Gross anatomy of the thyroid gland, anterior view

The thyroid gland weighs 6-20g depending on body weight, age, iodine and pregnancy status (Sari *et al.,* 2003; Hegedus *et al.,* 1983; Berghout *et al.,* 1987; Smyth *et al.,* 1997). It consists of follicles with no subdivisions and enveloped by a fibrous capsule.

The parathyroid glands are positioned on the posterior surface of the lateral lobes.

The blood supply of the thyroid gland is provided by the superior thyroid artery (branch of external carotid) and inferior thyroid artery (branch of thyrocervical trunk of the subclavian artery) bilaterally and usually a fifth artery the thyreoidea ima from the arch of the aorta enters in the midline. The blood supply to the thyroid gland accounts for 2% of the cardiac output although it only accounts for 0.4% of the body weight. The blood drains inferiorly into the internal jugular and the brachiocephalic vein.

1.1.2. Embryology of the thyroid gland

The thyroid gland is composed of follicular and parafollicular cells which are also called C-cells. The follicular cells form follicules of varying sizes, they secrete the thyroid hormones which are then stored in the lumen. The lumen contains homogeneous colloid, thyroglobulin (TG) and other proteins such as albumin. In between the follicles are the C-cells that secrete calcitonin in response to an increase in serum calcium levels. Calcitonin acts mainly be inhibiting bone resorption and therefore lowering serum calcium levels.

The thyroid gland is the first endocrine gland to develop on the 24th day of gestation (Trueba *et al.,* 2005). It originates from the endodermal epithelial cells on the median surface of the developing pharyngeal floor arising from the first pharyngeal arch. The C-cells arise from neural crest cells that migrated to the ultimobranchial body which then fuses with the thyroid gland. The timing of events is outlined below (Table 1.1).

If the above process fails and the thyroid gland does not form, congenital hypothyroidism occurs. Congenital hypothyroidism affects 1 in 4000 newborns, it is usually caused by thyroid dysgenesis (85%) and the other 15% is due to disorders of hormone synthesis (Trueba *et al.,* 2005). Thyroid dysgenesis is usually sporadic with only 2% having a positive family history (Castanet *et al.,* 2001). The main genes involved in dysgenesis are *PAX8*, *TITF1* and *FOXE1.*

Table 1.1. Timing of events during human thyroid development (Trueba *et al.,* 2005)

Gestational age	Anatomical or morphological events in thyroid development
22 days	Thickening of the floor of the primitive pharynx between the diverging aorta
26 days	Outgrowth and budding of the median thyroid primordium from the floor of the primitive pharynx. The inferior part of the fourth pharyngeal pouch forms the ultimobranchial body.
28 days	The median primordium grows caudally and appears bilobed. It is connected to the primitive pharynx by the thyroglossal duct.
32 days	Migration of the median primordium, still connected to the epithelium of the primitive pharynx
33 days	The thyroglossal duct starts to break down.
37 days	The median primordium consists of two lobes, an isthmus and a pedicle remnant. The continuity with the primitive pharynx is lost.
44 days	Median primordium fuses with the lateral components derived from the ultimobranchial bodies.
48 days	The thyroid reaches its final position in front of the trachea just inferior to the cricoid cartilage. It begins to form follicles.
10–12 wk	Follicles containing colloid become visible. The thyroid is able to incorporate iodine into thyroid hormones.

1.1.3. Thyroid physiology

The thyroid gland produces two principal hormones, thyroxine (T4) and triiodothyronine (T3) both dipeptides containing 4 and 3 iodine molecules respectively. Iodine supply is essential for the production of both hormones and they are the only iodine-containing compounds with established physiological significance. In the fetal life, this supply is received transplacentally and in the adult through absorption of Iodine from the intestine. Excess iodine can also cause hyperthyroidism and if occurs acutely then possibly hypothyroidism through the Wolf-Chaikoff effect by inhibiting hydrogen peroxide generation and therefore blocking TG iodination (Wolff and Chaikoff 1948). Therefore, there needs to be continuous monitoring to assess trends in iodine intake.

Iodine is converted to iodide before absorption in the small intestine and transported in the plasma to the thyroid gland. There it is actively transported by the sodium/iodine symporter across the basolateral plasma membrane of thyrocytes and trapped into the

follicular cell where its concentration is 20 to 50 times that of the serum (Figure 1.2; Berson and Yalow 1955) depending on thyroid stimulating hormone (TSH) stimulation and iodine intake.

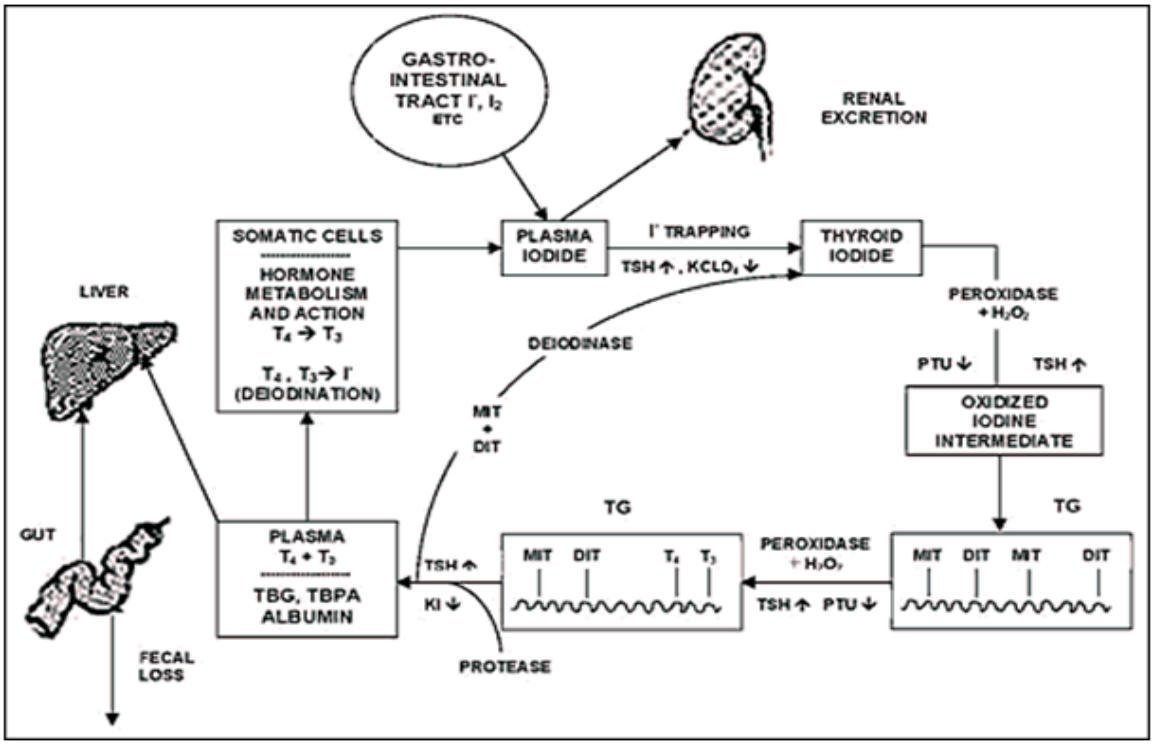


Figure 1.2. The iodide cycle. Ingested iodide is trapped in the thyroid, oxidized, and bound to tyrosine to form iodotyrosines in thyroglobulin (TG); coupling of iodotyrosyl residues forms T4 and T3. Hormone secreted by the gland is transported in serum. Some T4 is deiodinated to T3. The hormone exerts its metabolic effect on the cell and is ultimately deiodinated; the iodide is reused or excreted in the kidney. A second cycle goes on inside the thyroid gland, with deiodination of iodotyrosines generating iodide, some of which is reused without leaving the thyroid. Taken from www.thyroidmanager.org

TG is a glycoprotein secreted into the follicular lumen whose tyrosyls act as substrate for iodination and hormone formation. The iodide taken up by the cell is oxidized by thyroperoxidase (TPO) and then incorporated into TG to form monoiodotyrosine (MIT) and diiodotyrosine (DIT) which later form T3(MIT+DIT) and T4(DIT+DIT) (Virion *et al.,* 1981). This occurs at the apical plasma membrane-lumen boundary. When thyroid hormone is needed, TG is internalised at the apical membrane, transported in endosomes and lysosomes and broken down by protease to release T4 and T3.

Thyroid releasing hormone (TRH) is released from the paraventricular nucleus of the hypothalamus and it stimulates the release of TSH from the anterior pituitary gland. In hypothyroid patients, there is an increase in secretion and decrease in clearance of TSH (Ridgeway *et al.,* 1974). TSH is important in stimulating every step of thyroid hormone synthesis and secretion through the Gq/phospholipase C and cAMP cascade respectively (Song *et al.,* 2010). TSH binds to cell surface receptors to increase cAMP this stimulates the expression of iodine uptake transporters, TPO, TG and generation of hydrogen peroxide (H_2O_2) and increases the formation of T3 relative to T4, and internalisation of TG by thyrocytes through transcription factors such as TTF-1, TTF-2 and Pax-8.

Thyroid hormones in the blood stream are mostly bound to proteins with varying affinity. The 3 most common ones are, thyroxine-binding globulin (TBG), thyroid binding prealbumin (TBPA) and albumin. Approximately 99.98% of T4 is bound to 3 serum proteins: Thyroid binding globulin (TBG) ~75%; Thyroid binding prealbumin (TBPA or transthyretin) 15-20% and albumin ~5-10%. Only ~0.02% of the total T4 in blood is unbound or free (FT4) (Woeber and Ingbar 1968; Robbins 1992) and only ~0.4% of total T3 is free (FT3) (75% bound to TBG, ~5% to TBPA and 20% to albumin) in blood is free. The activity of thyroid hormones is determined by the unbound free fraction since only the unbound form enters the cell. The interaction of T4 and T3 with these proteins follows a reversible binding equilibrium. Thus, an increase TBG concentration will cause a shift of hormone from free to bound state and so the concentration of hormone will increase to restore the equilibrium and keep the free hormone levels the same in order not to alter the metabolic rate. If, however, there is a change in the total concentration of the hormone this will cause a change in the concentration of free hormone and so will affect the metabolic state of the patient. Serum T3 and T4 inhibit the release of TRH and TSH in a negative feedback cycle.

Three deiodinases (D1, D2 & D3) catalyze the generation and/disposal of bioactive thyroid hormone. They activate thyroid hormone by removing a single outer-ring iodine atom from T4 to form T3, an activating pathwaty (ORD) or inactivate thyroid hormone by removing a single inner-ring iodine atom from T4 to form rT3, an inactivating

pathway (IRD) (Bianco and Kim 2006; Bianco *et al.,* 2002). Normally one-third of T4 is converted to T3 and one-third is converted to rT3 and the remainder is metabolised by glucuronidation and sulfation. All family members of the deiodinases contain the novel amino acid selenocysteine (SeC) in their catalytic center. D1 is expressed mainly in the liver, kidneys and thyroid, it has ORD activity to produce peripheral T3 and clear rT3 but also has IRD activity to clear T4. D2 is mainly localised to the central nervous system and only has ORD activity, its role is to maintain tissue T3 levels with varying plasma T4 and T3. The role of D3 is to degrade thyroid hormone as it only has IRD activity and it is mainly expressed in the brain. However, it has been shown to be expressed in the placenta, uterus and fetal tissue possibly preventing excess levels of T3 in the fetus. However, it has been shown in mice animal models that these enzymes not only control the levels of thyroid hormones but also control their bioactivity (Ng *et al.,* 2004).

Most of the thyroid hormone actions are initiated by the binding of T3 to the nuclear receptors. There are 2 sources of intracellular T3, from plasma T3 or T3 locally produced from T4. In the liver and kidney for example, most the T3 is directly from plasma T3 however in the central nervous system a larger proportion of tissue T3 is locally produced from T4 (Marsili et al., 2011).

The levels of T4 and T3 in the brain are very well regulated by thyroid hormone serum levels, transport into cells and deiodinase expression. A study conducted on thyroidectomised rats and given a constant infusion of T4 to determine what doses are needed to normalise tissue T3 (Escobar-Morreale *et al.,* 1995). It showed that supraphysiological levels of serum T4 are needed to normalise tissue T3 in most organs except cerebral cortex and cerebellum where levels were normal over a wide range of T4 doses and independent of tissue T4. The maintenance of tissue T3 homeostasis over a wide range of T4 levels in the brain is partly explained by the down regulation of cerebral cortex 5'D-II with higher doses of infused T4 avoiding T3 excess (Escobar-Morreale *et al*., 1995). The same group showed that when thyroidectomised rats are given an infusion of T3, the level of tissue T3 in the cerebral cortex and

cerebellum are much lower at similar plasma concentrations of T3 (Escobar-Morreale *et al.,* 1999) indicating there is a preferential uptake of T4 by the brain.

Antithyroid antibodies

Characteristically pregnancy is a physiological state of lowered immune responsiveness, resulting in an improvement of most autoimmune conditions such as thyroid autoimmune disease. During this period there is an increase in regulatory $CD4^{+}CD25^{+}$ T-cells (Treg) which peaks in the second trimester mainly in the decidual tissue and peripheral blood, this is possibly due to fetal antigen presentation and oestrogen-induced expression of chemokines. These regulatory cells inhibit both Th1 (T helper cell-1 responsible for cellular immunity) and Th2 (T helper cell-2 responsible for humoral immunity) cells however there is a shift towards Th2 due to a lower sensitivity to Treg cells therefore avoiding the detrimental effects of Th1 which can cause fetal loss. During the couple of weeks before delivery the levels of Treg decline which results in a shift back to Th1 cells after delivery increasing the risk of autoimmune exacerbation 4-8 month after delivery such as postpartum thyroiditis (Saito *et al.,* 2010; Guerin *et al.,* 2009; Mjosberg *et al.,* 2007; Adams Waldorf and Nelson 2008; Weetman 2010; Galofre and Davies 2009).

Autoimmune thyroid disease is a spectrum of diseases that include Graves' disease, Hashimoto's thyroiditis and postpartum thyroiditis. The pathogenesis of autoimmune thyroiditis involves activation of the T-cell lymphocytes which in turn activate the B-cells to produce antibodies. These antibodies can either stimulate the TSH receptor such as anti-TSH receptor or block the production of thyroid antibodies such as anti-TPO antibodies and cause hyper- and hypothyroidism. They are also used to predict progression from subclinical to overt disease. The most prevalent antibodies in hypothyroid patients are anti-TPO and anti-TG antibodies and should be tested to identify autoimmune cause of hypothyroidism (American Thyroid Association 1995). Anti-TPO is more sensitive and specific (McLachlan and Rapoport 2004), however the prevalence of both these antibodies varies dramatically depending on the cut-off used for positivity. Anti-TPO is prevalent in ~53% of patients with Graves' disease and ~88%

of patients with Hashimoto's thyroiditis however on treatment levels can decrease by up 50% (Engler *et al.,* 1994). Anti-TG antibodies is prevalent in 50% of Graves' disease and 80-90% of patients with Hashimoto's thyroiditis (HT) (McLachlan and Rapoport 2004). Therefore, both these antibodies are generally used to screen for thyroid autoimmune disease and if positive further testing performed. During the course of pregnancy, levels of autoimmune thyroid antibodies decrease by an average of 60%, however even in the euthyroid women serum TSH levels are still relatively higher than controls throughout gestation (Glinoer *et al.,* 1994). It is important to note that autoimmune thyroid disease in pregnancy increases the risk of progression of thyroid disease 20 years later (Mannisto *et al.,* 2010).

1.1.4. Role of thyroid hormones

Thyroid hormones are important for development, growth and metabolism (Oppenheimer et al., 1987; Yen 2001) of all tissues. They regulate basal metabolic rate, increasing oxygen consumption in most target tissues, increase sensitivity of target tissues to catecholamines, thereby elevating lipolysis, glycogenolysis, and gluconeogenesis. The diverse features of hypo- and hyperthyroidism emphasize the different pathways and target organs these hormones act on. The major effects of thyroid hormone occur through nuclear receptors that mediate gene expression, however nongenomic actions also occur at the plasma membrane or cytoplasm (Cheng *et al.,* 2010) such as uncoupling oxidative phosphorylation, stimulation of energy expenditure by the activation of Na+-K+ ATPase activity, direct modulation of TH transporters and enzymes in the plasma membrane and mitochondria.

Biologically, some of the functions of TH include normal development of the skeletal system and musculature as well as being essential for normal brain development and regulates synaptogenesis, neuronal integration, myelination and cell migration. Based on studies on rats, the action of thyroid hormones on the brain involves oligodendrocyte differentiation, glial maturation in the cerebral cortex, cerebellum and hippocampus (Billon *et al.,* 2002; Martinez-Galan *et al.,* 1997).

The awareness of thyroid disease in the general population is rising as it is emerging as a potential contributor to morbidity from osteoporosis, hyperlipidaemia, hypercholesterolemia, hyperhomocysteinemia and cardiovascular and neuropsychiatric disease (Surks and Ocampo 1996; Helfand and Redfern 1998; Cooper 1998; Hak *et al.,* 2000; Morris *et al.,* 2001).

1.2 THYROID FUNCTION IN PREGNANCY

1.2.1 Changes during pregnancy

During pregnancy numerous physiological changes occur to provide the fetus with sufficient thyroid hormones, and these result in profound and complex effects on thyroid function (Figure 1.3). Firstly, the thyrotropic action of human chorionic gonadotrophin (hCG) results in low levels of TSH in the first trimester with a clear mirror image between the two. hCG is a glycoprotein hormone which consists of a 92 amino acid α-subunit and a 145 amino acid β-subunit coded by different chromosomes and are bound non-covalently before entering the circulation (Visconti and Zite 2012). The α-subunit is structurally homologous to that of TSH and their receptors are also analogous. The comparison was initially made when patients with trophoblastic tumours or hyperemesis gravidarum were reported to be hyperthyroid. hCG is produced by the gestational cytotrophoblasts which differentiate into extravillous cytotrophoblast and syncytiotrophoblast.

During implantation (first two weeks) the extravillious cytotrophoblast produces hyperglycosylated hCG which promotes invasion of the uterine wall, forming anchoring villi and increasing the circulation in the spiral arteries (Cole 2010, Sasaki *et al.,* 2008). The syncytiotrophoblasts form the epithelium lining the villous tree and produces regular hCG which maintains production of progesterone from corpus luteum until placenta takes over as well as promoting spiral artery angiogenesis.

The level of hCG peaks at 10 weeks when TSH reaches a nadir, the thyrotrophic impact seems to be the strongest at that gestation (Haddow *et al.,* 2008). The levels of hCG then decrease up to 20 weeks' gestation then reaches a plateau (Kennedy *et al.,* 2010). In twin pregnancies these levels are nearly double and the peak period lasts for a longer time (Madsen *et al.,* 2011).

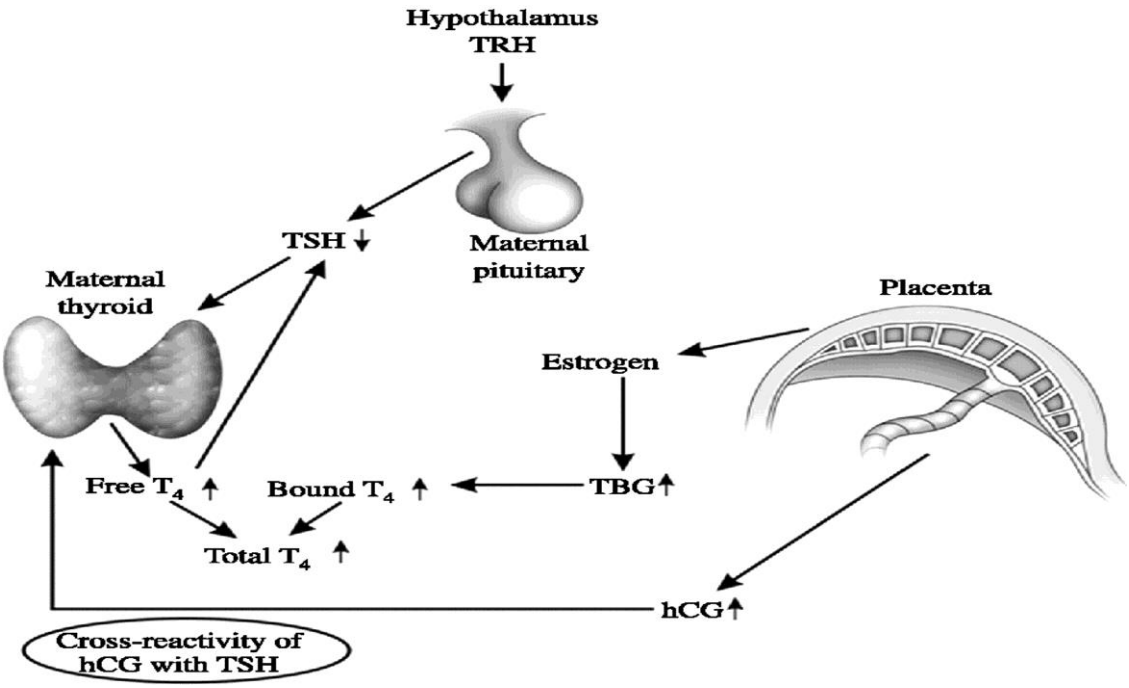


Figure 1.3. Thyroid physiological changes that occur during pregnancy. TRH, thyrotropin-releasing hormone; T4, thyroxine; TBG, thyroxine-binding globulin; hCG, human chorionic gonadotropin; PRL, prolactin). Taken from http://www.ejeonline.org/content/162/3/453/F4.expansion.html

The relationship between hCG and TSH is stronger at the lower TSH centiles possibly due to an adaptive mechanism where patients with higher TSH values need the thyroid gland to be stimulated by both TSH and hCG to produce adequate levels of thyroid hormones for the mother and fetus (Haddow *et al.,* 2008). The metabolism of hCG affects its thyrotropic activity where truncated β-hCG has higher thyrotropic potency than intact hCG (Yoshimura and Hershman 1995). However the stimulatory action of

hCG is relatively weak, a 10,000IU/L increment results in a mean FT4 increase of 0.6 pmol/L and lowering TSH by 0.1 mU/L (Glinoer *et al.,* 1990) therefore thyrotoxic levels are rare and probably restricted to the first trimester of pregnancy. After the hCG peak in the first trimester, TSH levels gradually increase and are highest in the 3rd trimester due to about a 30% reduction in free T4 and T3 in late pregnancy compared to values in earlier pregnancy (Glinoer *et al.,* 1990; Whitworth *et al.,* 1982). These changes in thyroid hormone levels in the 3rd trimester occur even in an iodine sufficient environment (Fister *et al.,* 2011). It is important therefore to use gestation specific reference ranges of thyroid hormones (Stricker *et al.,* 2007). Studies have shown that FT3 and FT4 levels start to increase 3-4 days postpartum (Kurioka *et al.,* 2005) however TSH levels start to decrease between 4 months to 1 year postpartum (Fister *et al.,* 2011; Soldin *et al.,* 2004).

During pregnancy there is a high level of oestrogen production reaching 60 mg/day during the last trimester (Katz and Kappas 1967). Estradiol more specifically has been shown to increase synthesis of TBG by the hepatocytes (Katz and Kappas 1967; Glinoer *et al.,* 1977) and it also increases sialylation of TBG (oligosaccharide modification) decreasing clearance (Bartalena 1990; Ain *et al.,* 1987). The increase in thyroid binding globulin (TBG) occurs throughout the first half of pregnancy, it plateaus at 24 weeks and stays unchanged until term (Skjoldebrand *et al.,* 1982; Glinoer *et al.,* 1990) however there is also an alteration in affinity for T4 due to a reversible change in structure that modulates thyroid hormone delivery to tissues (Zhou et al 2006). The high levels of TBG stimulate the production of thyroid hormones with an increase in total T4 and T3 however maintaining a normal active FT4 and FT3 levels in the first half of pregnancy (Glinoer *et al.,* 1990, Figure 1.3).

There is an increase in the level of TG during pregnancy with 2 out of 3 women having a significant increase from initial evaluation until delivery ($p<0.001$). Studies have not shown any correlation between TG and thyroid hormones, hCG or urinary iodine (Glinoer *et al.,* 1990) however there is a positive correlation with thyroid gland volume change in pregnancy.

As discussed above iodine is essential for the production of thyroid hormones. During pregnancy iodine requirements increase due to increased synthesis of thyroid hormones, an increase in iodide excretion due to increased renal blood flow and glomerular filtration rate and placental transfer and metabolism. The prevalence of Iodine deficiency in the population varies depending on location, a recent review of iodine status claims that approximately 70% of households worldwide have access to iodized salt (Zimmermann and Andersson 2012). Regions with greatest access are Western Pacific and Americas and those with least access are residing in the Eastern Mediterranean. The method that is mostly commonly used by the WHO to assess iodine status of a population is median urinary iodine concentration (UIC) since it reflects current iodine status and identifies acute changes in iodine intake (normal range for children and non-pregnant women 100-299 µg/l, and for pregnant women 150-249 µg/L). Surveys on UIC are available for 117 countries and show that 28.9% of the general population have low median UIC. However these surveys are done on children and non-pregnant women and do not truly reflect the iodine status of pregnant women or women of reproductive age (Zimmermann and Anderson 2012). Due to the physiological changes during pregnancy UIC is not a good indicator of iodine status especially in the first trimester of pregnancy when there is an increase in glomerular filtration rate and therefore increased excretion of iodine even when iodine deficient. During the later stages in pregnancy, placental and fetal demand of iodine also increase.

A recent study was conducted to assess whether median UIC in school children and non-pregnant women can be used to predict iodine status of pregnant women (Wong *et al.,* 2011). This study showed that when median UIC was adequate or above requirements in school aged children or non-pregnant women, approximately 50% of the time pregnant women were iodine deficient. Body reserves are reduced by 40% during pregnancy and therefore women during this period are more susceptible to iodine deficiency even in iodine replete areas. Marchioni *et al.,* measured mean UIC in 51 pregnant women and 100 matched controls and showed that despite adequate supplementation 92% of pregnant women were iodine deficient compared to 4% in the control group (Marchioni *et al.,* 2008). Another group in France reported that the

prevalence of maternal iodine deficiency defined as UIC < 150 is 66.3% in the first trimester and 85.8% in the 3rd trimester (Brucker-Davis *et al.,* 2012; Hiéronimus *et al.,* 2009), the reduced rate in the first trimester is likely due to the increased excretion rate of iodine early in pregnancy. Other studies however suggested that the increase in excretion rate of iodine is stable through all 3 trimesters (Beckers 1991; Smyth e*t al.,* 1997). The world health organisation (WHO) currently recommends iodine supplementation in countries with iodine deficiency for women of child-bearing age (150 µg/day or annual dose of 400 mg) and pregnant and lactating women (250 µg/day or annual dose of 400 mg).

Thyroid gland volume has been used to reflect long-term iodine status in a population; it is thought to develop due to the increased TSH stimulation of the gland in response to reduced thyroid hormone production. Until recently volume has been measured visually or by palpation. The WHO described 'goitre' as a thyroid gland with lobes larger than the terminal phalanges of the thumb. Recently ultrasound has been used to establish normal ranges of thyroid volume in iodine-replete areas in 6-12 year old children (Zimmermann *et al.,* 2004) and this can be used to describe prevalence of goitres more accurately in a population. Thyroid gland size is generally related to genetic factors, sex, age, TSH, FT4, smoking, body weight, height, waist-hip ratio, body surface area, total body water, fat mass and body fat (Gomez *et al.,* 2000; Hansen *et al.,* 2004). In the nonpregnant and pregnant women, size increased with BMI (Hansen *et al.,* 2004; Hegedus 1990; Fister *et al.,* 2009).

During pregnancy thyroid volume was inversely correlated with TSH as with the general population (Gomez *et al.,* 2000) and positively correlated with TG levels and T3/T4 ratio (Glinoer *et al.,* 1990), but there was no correlation with urinary iodine concentration (Gomez *et al.,* 2000). There is conflicting evidence as to whether thyroid volume increases during pregnancy (Azizi *et al.,* 2003; Berghout and Wiersinga 1998; Fister *et al.,* 2009; Nelson *et al.,* 1987; Brander and Kivisaari 1989). The proposed reasons why thyroid gland volume increases during pregnancy are due to the increase vascularity (Rasmussen *et al.,* 1989; Fister *et al.,* 2009), increase total body water (Gomez *et al.,* 2000), stimulation by hCG and iodine deficiency (Glinoer *et al.,* 1990)

and therefore normal ranges of thyroid gland volume need to be established for pregnant women.

1.2.2. Fetal thyroid function

The lack of thyroid hormones during fetal growth and childhood results in prominent abnormalities. This is illustrated in cretinism in an iodine-deficient population, with evidence of restricted physical and mental development, reduced muscle tone or spastic diplegia, squint, thickened skin, enlarged tongue, protruding abdomen.

There are three phases of fetal thyroid development; Embryogenesis, hypothalamic maturation and development of hypothalamic-pituitary-thyroid system control. Human studies have shown that the fetal thyroid gland begins to produce thyroxine at 10-12 weeks gestation (Shepard 1967). However there is conflicting evidence as to the gestation at which functional maturation of the fetal pituitary-thyroid axis is achieved. Some authors suggest that TSH secretion is responsive to changes in serum FT4 as early as 11 weeks (Greenberg *et al.,* 1970) while others suggest that such maturation occurs largely during the last half of pregnancy (Fisher *et al.,* 1970; Fisher *et al.,* 1973, Fisher *et al.,* 1977).

The amount of thyroid hormones in amniotic fluid was not found to correlate with the fetal or maternal thyroid hormone serum levels (Pekonen *et al.,* 1984). After the introduction of cordocentesis it became possible to examine the fetal pituitary and thyroid development under physiological conditions (Nicolaides *et al.,* 1986). A study by Ballabio *et al.,* used cordocentesis samples of 23 women and examined fetal thyroid function from 18-31 weeks' gestation, demonstrating an increase in fetal serum TSH, T4 and FT4 throughout gestation. Compared to the maternal serum levels, fetal TSH was higher and T4 was lower however FT4 reached maternal levels by 28 weeks gestation. The threshold for negative feedback control seems to be set at a higher level, however these small number of cases may not be truly representative of

physiological levels especially that the majority of them were severely anaemic due to red cell isoimmunisation (Ballabio et al., 1991).

In another study which included 62 fetuses where cordocentesis was performed for prenatal diagnosis and the fetuses were found to be normal, there was a significant increase in the concentration fetal serum TSH, TBG, T4, FT4, T3 and FT3 with gestation and no association between fetal and maternal thyroid hormones and TSH concentrations (Thorpe-Beeston *et al.,* 1991a).

This suggested that the fetal thyroid axis develops independently of the mother's thyroid axis. There was no significant association between fetal TSH, TBG and thyroid hormones. Although the total and FT4 concentrations reached adult levels by 36 weeks' gestation in this study, the total and FT3 were always less than 50% of mean adult concentrations, which possibly reflects the fact that in the fetus the mechanism of conversion from T4 to T3 is immature or lacking. Fisher *et al.,* also showed that there is 10 fold increase in the ratio of serum T3 to T4 from 30 weeks to 1 month postnatally (Fisher *et al.,* 1977). Possible explanations are late maturation of the pituitary thyroid axis or rapid deiodination by the placenta. The increase of fetal thyroid hormones with no correlation to TSH suggests that the thyroid gland matures independently of TSH, and possibly improved transfer via the placenta' however this was not supported by another study (Vulsma et al., 1989). The continued increased of TSH in the 3rd trimester even with normal FT4 levels may indicated that FT3 is more important for negative feedback in the fetus.

1.2.3. Transfer of maternal thyroid hormones the fetus

The importance of the maternal thyroid hormones for the fetus were initially demonstrated by Morreale de Escobar and colleagues on pregnant rat animal models where they performed thyroidectomies on rats and compared thyroid hormone levels in the mother and the embryo to those of control rats (Morreale de Escobar *et al.,* 1985). They showed that thyroidectomy reduced the reproductive capacity and number of

viable embryos. They also demonstrated that the weight of the embryotrophoblast in the rats with a thyroidectomy was reduced throughout pregnancy compared to those of controls and that the weight of individual organs such as brain, liver and lung of the fetus was reduced at the end of pregnancy (20 and 21 days gestation) (Morreale de Escobar *et al.,* 1985).

It was initially thought that the placenta was impermeable to thyroid hormones until early studies have detected T4 and T3 in 9- to 14-day old rat embryos before the onset of fetal thyroid gland function (Sweney and Shapiro 1975; Obregon *et al.,* 1984; Woods et al 1984). In humans the landmark study by Vulsma *et al.,* which studied 25 neonates with a complete organification defect or thyroid agenesis and measured their cord blood at birth, found T4 levels ranging from 35-70 nmol/l (Vulsma *et al.,* 1989). Since then, T4 was found to be present in fetus from as early as 4 weeks gestation (Obregon *et al.,* 2007). A study examining the expression of thyroid hormone transporters in 110 normal human placentas, showed that mRNA encoding thyroid hormone transporters are expressed from 6 weeks and throughout gestation (Loubiere *et al.,* 2010). The human fetus is dependent on transplacental delivery of maternal thyroid hormones before 16 weeks gestation (Patel et al., 2011).

The placenta acts as a barrier protecting the fetus from changes in maternal thyroid function and transfer is carefully regulated by trophoblast cell membrane transporters, placental deiodinases D3 and D2. Thyroid hormones are also metabolised in the placenta as both D3 and D2 deiodinases are expressed from the first trimester of pregnancy and their expression falls as gestation progresses. Placental D3 activity however is approximately 200 times that of D2 (Patel *et al.,* 2011), they inactivate T4 to rT3 and activate T4 to active T3 respectively. The role of D2 in the placenta is likely only for housekeeping purposes whereas D3 prevents excess T4 reaching the fetus.

It has been suggested that the main thyroid hormone binding protein produced and expressed in the placenta is transthyretin (TTR) (McKinnon *et al.,* 2005) and that maybe is how thyroid hormones are transported in the placenta.

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1.3 THYROID DYSFUNCTION

1.3.1. Clinical hypothyroidism due to iodine deficiency

Worldwide iodine deficiency is an important cause of hypothyroidism and studies have shown a correlation between iodine deficiency and raised TSH levels in newborns (Delange *et al.,* 1986; Zimmermann *et al.,* 2005). A study conducted in Switzerland showed that an increase in iodine concentration in salt by 25% from 15 to 20 mg/kg results in an increase in median UIC in pregnant women from 138 µg/L in 1998 to 249 µg/L in 2004 ($p<0.01$) and a reduction in number of newborns with TSH >5 mU/L from 2.9% in 1992-1998 to 1.7% in 1999-2004 (Zimmermann *et al.,* 2005). Studies on rat animal models have shown that iodine deficiency does initially result in reduced levels of T4 and increased levels of TSH and thyroid volume however T3 levels did not change significantly (Minato *et al.,* 2012) and the metabolism of T4 is not altered in an iodine deficiency state. It is therefore likely that the level of T3 is maintained by preferential biosynthesis of T3 over T4 in the thyroid gland. This seems reasonable as T3 is metabolically more active and has fewer iodine atoms. The level of serum T3 was only affected when the T4 level was reduced to 5% of normal value (Pedraza *et al.,* 2006) and serum T3 must decrease before many T3-dependent tissues become T3-deficient. Thyroid autoregulation is therefore important in adapting to iodine deficiency preventing the decrease of T3 in serum and most tissues despite a significant reduction of serum T4. However the brain's supply of thyroid hormones is mainly from the conversion of T4 to T3 by its own deiodinase than from circulating T3 and therefore a reduction in serum T4 would pose a potential risk to the developing brain (Calvo *et al.,* 1990).

The effects of Iodine deficiency on the pregnancy and the fetus have been well documented (Gardner 1975) and varied depending on the severity of Iodine deficiency. Iodine deficiency causes endemic hypothyroidism as both mother and fetus will be deficient throughout the pregnancy whereas sporadic hypothyroidism which is only fetal in origin. The most severe form of congenital hypothyroidism results in cretinism

its features include deaf-mutism, short stature, spasticity and profound mental retardation. The association between maternal iodine deficiency and neurodevelopmental delay in the offspring has been recognised over a 100 years. Iodine deficiency is the single most important preventable cause of brain damage worldwide (World health Organisation, WHO). People living in areas affected by severe iodine deficiency may have an intelligence quotient (IQ) of up to 13.5 points below that of those from comparable communities in areas where there is no iodine deficiency (Bleichrodt and Born 1994). This mental disability has an immediate effect on child learning capacity, women's health, the quality of life in communities, and economic productivity. Therefore universal salt iodization was introduced and is aimed to iodize all salt for human and animal consumption to the internationally agreed recommended levels.

Studies are now linking iodine deficiency with adverse pregnancy outcomes, recently a study examined study examining the effects of iodine supplementation during the second half of pregnancy showed that pregnancies supplemented with iodine were less likely to deliver preterm and the neonate was less likely to be growth restricted (Joshi *et al.,* 2011). Another preliminary study looking at the effects of maternal iodine supplementation (300µg) during the first trimester of pregnancy on psychological development of infants aged 3 to 18 months (Velasco *et al.,* 2009), demonstrated an improved psychometric assessment (higher psychomotor development index, p=0.02 and behavioural rating score).

1.3.2. Clinical hypothyroidism without iodine deficiency

The prevalence of overt hypothyroidism in pregnancy is 0.3% (Klein *et al.,* 1991). The most common cause of hypothyroidism in an iodine sufficient population is HT. HT is a cell-mediated immune response that results in the gradual destruction of thyroid tissue with characteristically positive anti-TPO and anti-TG antibodies although they don't play a significant role in the pathogenesis. More details in section 1.3.4.

The endocrine society clinical practice guidelines recommend when treating overt hypothyroidism the dose of levothyroxine should be titrated aiming for a TSH <2.5 mIU/L in the 1st trimester and <3 mIU/L in the 2nd and 3rd trimesters (De Groot *et al.,* 2012) or using trimester specific reference ranges established in the laboratory. The thyroid function testes should be repeated every 4-6 weeks.

Adverse effect on neurodevelopment in children

The initial mention of non-iodine hypothyroidism measured using serum butanol extractable iodine and the effects of this on neuropsychological development of the offspring was initially mentioned in 1971 (Man *et al.,* 1971). In 1999 Pop et al., measured FT4, TSH and Anti-TPO antibody levels at 12 and 32 weeks gestation in 220 pregnant women who did not have a history of thyroid disease living in an iodine sufficient area and who had a live born which was phenotypically normal with no complications such as prematurity, birth weight above 2500 grams and did not develop pre-eclampsia. The thyroid function was then correlated with the child's development at 10 months of age using the Dutch version of the Bayley Scales of Infant Development (Bayley, 1969) performed by a blinded psychologist. The 10 month gestation was used to avoid later bias from environmental factors such as psycho-social aspects (Pop *et al.,* 1999). In the women with FT4 levels below the 5th (<9.8pmol/l) and 10th (<10.4pmol/l) percentile at 12 weeks, their offspring scored significantly lower in the Bayley psychomotor subscale (mean difference 14.1 and 7.4 respectively) this was not evident at 32 weeks gestation. A difference of 10 points on the psychomotor development index is equivalent to 1 month delay in development (Bayley, 1969). They showed that the lower the FT4 levels the lower the psychomotor development index scale (linear regression R 0.46, p=0.03). All the children they assessed had normal T4 and TSH values one week after birth as examined by the national screening programme for congenital hypothyroidism. This was the first study to show that low FT4 levels <10th percentile at 12 weeks was associated with an increased risk of impaired neurodevelopment in the child at 10 months of age (RR 5.8, 95% CI 1.3-12.6). This study also demonstrated that women that were Anti-TPO antibody positive at 32

weeks, 46% of them had FT4 levels below the 10th centile at 12 weeks and therefore this might provide an explanation of the result of their previous studies (Pop *et al.,* 1995, details below) which showed the positive Anti-TPO antibodies at 32 weeks was associated with impaired child development at 4.5 years of age (decrease in IQ of 10 points).

Controversially, screening for subclinical hypothyroidism started after the publication of an American study of 25,216 women linking high levels of TSH (combined with lower levels of FT4) in the second trimester and lower IQ scores in the offspring (Haddow et al 1999). Women were divided into 2 groups, those with TSH levels >99.7th percentile (47 women) and those with TSH between 98th-99.6th percentile (15 women). Serum T4, FT4 and anti-TPO were measured in these 62 women (15 had already been diagnosed with hypothyroidism and 14 were on thyroxine) and 124 matched controls. The mean FT4 in the 62 affected women was 9.13 pmol/L (vs 12.5 pmol/L in the control group) classifying this group as overt rather than subclinical hypothyroidism. The neuropsychological testing was performed by 2 blinded psychologist, at 7 and 9 years of age at the time of assessment. The average IQ score was 7 points lower and 19% of children of women with hypothyroidism had scores $\leq$85 vs 5% of the control children. The high percentage of anti-thyroid peroxidase antibody positivity (77%) in hypothyroid women may indicate that the cause of hypothyroidism in these women is chronic autoimmune thyroiditis however the threshold in this study for positivity was low (>2 U/mL). Follow up of these women 11 years later identified that 4% of the control women were diagnosed with hypothyroidism compared to 64% of those that they identified during pregnancy. They advocate for screening as not only will it improve the childs IQ score by 4 but also will treat the women early and reduce morbidity as 64% were later diagnosed with the condition.

Obstetric complications

Overt hypothyroidism is commonly associated with anovulation due to hyperprolactinemia from increasing TRH serum levels. Therefore, pregnancy in untreated women is unlikely and if it does occur the risk of miscarriage is high

(Abalovich *et al.,* 2002). Numerous studies reported on the association of overt hypothyroidism and adverse pregnancy outcome; however it seems that with thyroid hormone replacement the pregnancy outcome can be good (Greenman *et al.,* 1962; Pekonen *et al.,* 1984). The endocrine society clinical practice guidelines state that there is good (level A) evidence to show that overt hypothyroidism can have serious adverse effects on pregnancy (De Groot *et al.,* 2012).

An early study published in 1962, studied 23 pregnancies with suspected thyroid disorder assessed by butanol-extractable iodine (BEI) (normal range for pregnancy 5.5-10.5 µg/100ml) which is a measure of thyroid function (Greenman *et al.,* 1962). The outcome included 1 miscarriage, 2 fetal deaths, 2 neonatal deaths, 5 infants were assessed to be severely mentally disabled at about 1 year of age (3 of them were born premature, 1 was growth restricted, 1 had a severe heart condition). This study however demonstrated that those women who were hypothyroid not on treatment with low BEI had much worse outcomes than those who are clinically hypothyroid but with normal BEI as well as those with known hypothyroidism on inadequate treatment (BEI is low) (Greenman *et al.,* 1962).

Montoro et al., (1981) is one of the earliest reports on the outcome of 11 pregnancies with biochemically confirmed hypothyroidism. The thyroid function tests were performed across gestation includes FT3, FT4 and TSH and hypothyroidism was defined as TSH >5 mU/ml and T4 <4.5 µg/dL. One patient developed PE and IUFD occurred at 29 weeks, there was no spontaneous preterm labour, none of the neonates weighed <2500g, one baby had Down syndrome otherwise there was no developmental delay in milestones in the first 2-3 years (Montoro *et al.,* 1981). Another report of 28 pregnancies, 16 diagnosed with overt hypothyroidism, defined as TSH> 10 mIU/mL and T4 < 4.5 µg/dL (nonpregnant range used); (Davis *et al.,* 1988). Women with overt hypothyroidism were more likely to develop PE (44%, 7/16), fetal death (19%, 3/16), preterm delivery <37 weeks (mean gestational age 35.2 weeks) however most were induced due to PE, 1/16 was spontaneous preterm labour) and have a birth weight <2500g (31%, 5/16 pregnancies however 3/16 were due to induced preterm labour for PE and 1 was due to spontaneous preterm labour and the other was growth

restricted at 38 weeks). During repeat pregnancies, 5 of these women where followed up and given LT4 and were clinically and biochemically euthyroid throughout, 4 had low birth weight infants due to premature rupture of membranes in 3 cases and PE in 1 case however these women were high risk women to previous premature deliveries and still births (Davis *et al.,* 1988). Due to small number in these reports, conclusions based on statistical analysis are not informative.

The evidence linking overt hypothyroidism and PE is still not consistent. A prospective study of 23 women with overt hypothyroidism (clinically and biochemically) showed an increased risk of PE and gestational hypertension, with 22% developing either (vs 7.6% in the general population, $p<0.04$). They also were more likely to deliver infants <2500g (22 vs 6.8%; $p<0.02$) this was due to the increased prematurity due to gestational hypertension. There was no increase in spontaneous preterm labour. One of these women has a still born and developed eclampsia (Leung *et al.,* 1993).

A Finnish study found that women who have overt hypothyroidism in the 1st trimester of pregnancy were not at an increased risk of developing PE (1.9% in both groups) (Mannisto *et al.,* 2010). This was consistent with a study by Negro et al., which also showed no association between hypothyroidism and pregnancy-induced hypertension (Negro *et al.,* 2010a).

A retrospective study of 150 pregnancies, with known overt hypothyroidism, on levothyroxine were examined to correlate thyroid function at conception with pregnancy outcome (Abalovich *et al.,* 2002). These include women with autoimmune thyroid disease. Overt was defined as TSH >5 mIU/L and FT4 <4.5 µg/dL and subclinical when TSH >5 mIU/L and FT4 within the normal range however both groups were combined as the hypothyroid group. At conception 99 were euthyroid, 16 with overt hypothyroidism and 35 with subclinical hypothyroidism. The miscarriage rate was higher in the hypothyroid group (31.4 vs 4%, $p<0.0001$) however all the miscarriages occurred in those that were treated inadequately. The term delivery rate was lower in the hypothyroid group (58.8 vs 84.5%, $p=0.18$) however the likelyhood of preterm delivery is higher if treatment was inadequate (20.8 vs 92.6% term delivery rate). This

study showed that the adverse outcomes were not related on the severity of the disease but on the adequacy of treatment (Abalovich et al., 2002). This study highlights the need to monitor these patients carefully throughout pregnancy.

Effects of treatment

A recently published study, however, has looked at the effect of treatment of hypothyroid women and the children's IQ score at 3 years of age (Lazarus et al 2012). They recruited 21,846 women with singleton pregnancies before 15^{+6} gestational weeks (median 12^{+3} weeks) with no history of thyroid disease and randomised them into screening group (10,924 women) and control group (10,922 women). A blood sample was taken from both groups, the sample was analysed for TSH and FT4 within 1 week for the screening group and after delivery for the control group. The definition of screen positive was TSH > 97.5^{TH} percentile and FT4<2.5^{TH} percentile or both. Women that were screen positive were started on 150 micrograms of levothyroxine and levels were checked 6 weeks after levothyroxine initiation and at 30 weeks to adjust dose as necessary for a target TSH level of 0.1-1.0 mIU/L. The primary outcome of the study was the IQ at 3 years of age in women who were screen positive. The children were assessed by the Wechsler Preschool and Primary Scale of Intelligence, 3^{rd} edition (2003) by a blinded psychologist. Based on the Haddow et al (1999) they expected a decrease in the percentage of children with an IQ score less than 85 at 7 years of age (5% in the screened vs 15% in the control). In the screened group, 4.6% were screen positive and vs 5% in the control group. Treatment in the screened group was started at a median gestational age of 13^{+3} weeks. Psychological testing was completed in 78.2% in the screened who tested positive and 73.3% in the control group who tested positive. The mean standard IQ score was 100 in the control and 99.2 (p=0.40) in the screened group. The percentage of children with an IQ <85 was 12.1% in the screened and 14.1% in the control group. This difference was not significant (p=0.39) and there was no association between initial TSH measurement and IQ score of the children at 3 years of age. This study supports the current

American Thyroid Association guidelines stating no evidence for routine screening in pregnancy.

1.3.3 Clinical hyperthyroidism

The prevalence rate of overt hyperthyroidism in pregnancy is 0.1-0.4%. Graves' disease is the most common cause of hyperthyroidism in females during the reproductive years accounting for 85-90% of causes of overt hyperthyroidism in pregnancy (Galofre and Davies 2009). Hyperthyroidism in pregnancy that is not treated adequately increases the risk of preeclampsia, congestive heart failure, thyroid storm, miscarriage, stillbirth, preterm delivery and placental abruption. Fetal hyperthyroidism which occurs in <0.01% of pregnancies may result in tachycardia, fetal goitre, accelerated bone maturation, growth retardation, low birth weight and malformations (Zimmerman 1999) and is either caused by maternal thyroid hormones or the stimulating thyroid receptor antibodies crossing the placenta which have an effect on the fetal thyroid gland after 12 weeks gestation (Chan and Mandel 2007; Laurberg *et al.,* 2009). Neonatal hyperthyroidism occurs in 5% of newborns to mothers with Graves' disease and persists for 12 weeks as the maternal antibodies have a half-life of 3 weeks (Chan and Mandel 2007; Laurberg *et al.,* 2009).

The aim of treatment during pregnancy is maintain FT4 at or just above the upper limit of the nonpregnant reference range (De Groot *et al.,* 2012). It is recommended to treat using propylthiouracil in the first trimester (to avoid the association of methimazole with specific congenital abnormalities) and methimazole in the 2nd and 3rd trimesters (to reduce the risk of liver toxicity with propylthiouracil. If however the patient develops adverse reactions to these drugs, or requires high doses or is non adherent with treatment and have uncontrolled hyperthyroidism then a subtotal thyroidectomy may be indicated in the second trimester. There is no evidence treatment of subclinical hyperthyroidism improves pregnancy outcome and can negatively affect fetal outcome (De Groot *et al.,* 2012)

A meta-analysis of clinically hyperthyroid patients from 8 studies examining the effect of treatment (propylthiouracil and methimazole) on pregnancy outcome showed a

reduced risk of preterm delivery (RR 0.23, 95% CI 0.1-0.52), PE (RR 0.23, 95% CI 0.06-0.89) and low birth weight (RR 0.38, 95% CI 0.22-0.66) (Vissenberg et al., 2012).

1.3.4. Euthyroid autoimmune thyroiditis

In a survey done on 13,344 disease free individuals in the United States population, has shown that anti-TG antibodies were positive in 10.4 +/- 0.5% and anti-TPO antibodies in 11.3 +/- 0.4% (Hollowell *et al.,* 2002). Prevalence of antibodies was higher in women than men, increases with age, more prevalent in whites (12.3 +/- 0.5%) than blacks (4.5 +/- 0.3%) ($p < 0.001$). In this study anti-TPO antibodies were significantly associated with hypo- or hyperthyroidism, but anti-TG antibodies were not (Hollowell *et al.,* 2002). The overall prevalence of anti-TPO in disease-free females in the US was 14.6% and that of anti-TG was 13.8% (Hollowell *et al.,* 2002). In a large prospective study of 2227 caucasian pregnant women the prevalence anti-TPO antibodies in the euthyroid women is 7.9% (169/2143) (Negro et al., 2007a). Another population based cohort of euthyroid pregnant women in Finland described the prevalence of anti-TG antibodies to be 3.6% and the prevalence of anti-TPO antibodies was 3.0%, lower than previously reported however only women with TSH and FT4 within the normal range were included (Mannisto *et al.,* 2012). A Dutch study screened 9778 pregnant women and found the prevalence of anti-TPO to be 4.7% (Ghassabian et al., 2012). The reason for this discrepancy is due to the fact that the cut-off used for positivity varies significantly.

There are some data showing that the higher the level of iodine intake the higher the prevalence of thyroid antibodies (Hollowell *et al.,* 2002; Kasagi *et al.,* 2009; Aghini-Lombardi *et al.,* 1999) and in a population previously iodine-deficient given iodine prophylaxis the incidence of thyroid autoimmunity increased 4 fold (Heydarian *et al.,* 2007). Although one study did link high iodine intake with a higher rate of postpartum thyroiditis (Guan *et al.,* 2005), most studies did not find an association and iodine

supplementation is considered safe in pregnancy even if the woman was anti-thyroid antibody positive (Antonangeli *et al.,* 2002; Nohr *et al.,* 2000).

The latest endocrine society clinical practical guidelines recommend that autoimmune euthyroid women at the early stages of pregnancy are at risk of developing hypothyroidism and should therefore be monitored every 4-6 weeks for raised TSH above reference range (De Groot *et al.,* 2012).

Adverse effect on neurodevelopment in children

The study by Matsuura and Konishi in 1990 was the first to document that the fetal brain development is adversely affected when both mother and fetus are hypothyroid due to chronic autoimmune thyroiditis (Matsuura and Konishi 1990). However the data regarding the effect of maternal anti-thyroid antibody on the fetal neurodevelopment is not consistent. A study by a Danish group measured TSH, FT4, FT3 and Anti-TPO (titre >100U/ml) antibodies at 32 weeks gestation and 4 weeks postpartum and thereafter at 6 weekly intervals until 34 weeks postpartum in 230 women (Pop *et al.,* 1995). The child's development was assessed by the Dutch translation of the McCarthy Scales of Childrens Abilities (MSCA). They defined thyroid dysfunction as abnormal TSH (normal range 0.14-3.5mU/L) and FT4 (normal range 8.9-18pmol/L), and that of subclinical hypothyroidism as high TSH with a normal FT4. They described an association between Anti-TPO antibody positivity at 32 weeks gestation and lower MSCA assessment scores in 4 of the 6 categories at preschool age, most significantly in the cognitive scores with a 10.5 point difference. All the women had normal FT4 and TSH levels at 32 weeks gestation. However to correct for any possible confounding factors such as maternal depression, they performed a logistic regression analysis and demonstrated that Anti-TPO antibodies at 32 weeks gestation, low educational level of mother, current major maternal depression, previous episode of depression in mother's life, family history of depression were all factors associated with lower cognitive (GCS) score in the offspring. This study has shown that euthyroid women with Anti-TPO antibodies is associated with neurodevelopmental delay (Pop *et al.,* 1995). This was consistent with a study from China which measured serum TSH, FT4 and anti-TPO antibodies in 1268 pregnant women at 16-20 weeks gestation (Li *et al.,* 2010). Patients

who were euthyroid with positive anti-TPO antibodies had a lower mean intelligence score by 10.56 points (p=0.001) and lower mean motor scores by 9.03 (p<0.001) than matched controls at 25-30 months of age. Using a multivariate logistic regression analysis they found an independent effect of anti-TPO antibodies and increased serum TSH on intelligence (OR 6.69, OR 15.63 respectively) and motor scores (OR 9.23, OR 8.25 respectively).

However, the same group (Pop *et al.,* 1999) then measured Anti-TPO antibodies at 12 and 32 weeks gestation in 220 pregnant women who did not have a history of thyroid disease living in an iodine sufficient area and who had a live born which was phenotypically normal with no complications such as prematurity, birth weight above 2500 grams and did not develop pre-eclampsia and assessed the offspring's psychomotor development at 10 months of age. They showed using multiple logistic regression that Anti-TPO antibody levels >100U/ml at 12 and 32 weeks gestation was not an independent predictor of the childs psychomotor development at 10 months of age. Some of these women had low FT4 (<10^{th} centile for gestation) and therefore were not euthyroid. The cause of discrepancy in results possibly could be due to the fact that the study by Pop *et al.,* 1999 examined the offspring at 10 months of age which may be too early to notice a change in development.

Obstetric complications

We have shown above the association between clinical hypothyroidism with adverse pregnancy outcome. These adverse outcomes could be the result of the deficiency of thyroid hormones or could be the result of increased prevalence of anti-thyroid antibodies in these conditions. The prevalence of these antibodies are also higher in infertile women (Poppe and Velkeniers 2003a). A meta-analysis performed to establish whether anti-thyroid antibodies are associated with various adverse pregnancy outcomes (van den Boogaard *et al.,* 2011) found women that were anti-thyroid antibody positive (334 women) were more likely to be subfertile (OR 1.5, 95% CI 1.1-

2.0; Figure 1.5). However, no association was found between the presence of thyroid antibodies and the clinical pregnancy rates after IVF (OR 0.67, 95% CI 0.36-1.4). Studies are now examining whether anti-thyroid antibody positive euthyroid women who managed to get pregnant are at risk of developing adverse pregnancy outcomes.

The first study that linked thyroid autoantibodies to risk of miscarriage showed that in 552 women who presented in the first trimester of pregnancy, there was an increased risk of miscarriage in those with either anti-TPO and anti-TG antibody positivity (Stagnaro-Green *et al.,* 1990). The rate of miscarriage is 8.4% in the antibody negative women and 17% in the antibody positive women. They also showed that thyroid hormone levels are not associated with the rate of miscarriage. Glinoer *et al.,* 1994 also showed that euthyroid women with positive anti-thyroid antibodies had and increased risk of miscarriages (7% vs 3.3%) however due to small numbers this was not statistically significant. Important to note is that the rate of premature delivers doubled (16% vs 8%, $p<0.005$) although the level of anti-thyroid antibodies decreased during pregnancy by an average of 60% (Glinoer *et al.,* 1994). However this group did not use gestation specific normal range of TSH, the upper limit of normal TSH was 4 mU/L. There results reaffirmed their previous results published in 1991, which demonstrated that euthyroid patients with positive anti-thyroid antibodies had an increased risk of miscarriage (13.3% vs 3.3%, $p< 0.001$) (Glinoer *et al.,* 1991). Thereafter numerous studies looked at pregnancy loss and preterm delivery in anti-thyroid antibody positive women examining specific groups such as those experiencing recurrent pregnancy loss and undergoing assisted conception.

A summary of the literature concerning anti-thyroid antibodies and miscarriage rate was shown and a meta-analysis performed of both case-controlled and longitudinal studies from 1990 to 2003 (Prummel *et al.,* 2004, Table 1.2). They found a clear association between anti-thyroid antibodies and miscarriage (OR 2.73, 95% CI 2.2-3.4 in case-control studies; OR 2.30; 95% CI, 1.80–2.95 in longitudinal studies). Some possible explanations for this include, the fact that there is a heightened autoimmune state rejecting the fetal allograft, or the fact that these antibody positive women were slightly older (mean age difference 0.7 +/-1.0 years, $p<0.001$) or due to thyroid failure

and higher TSH levels in the antibody positive but euthyroid women (difference 0.81 +/-0.58, p=0.005).

Table 1.2. Meta-analysis of prospective studies analyzing abortion rates among women with antithyroid antibodies versus women without antibodies.

Author	Abortion rate		OR (95% CI)
	Antibody negative	Antibody positive	
Stagnaro-Green *et al.* 1990	17/100(17 %)	33/392 (8 %)	2.23 (1.19–4.20)
Glinoer *et al.* 1991	6/45 (13 %)	20/603 (3 %)	4.48 (1.70–11.81)
Lejeune *et al.* 1993	5/23 (22 %)	16/340 (5 %)	5.63 (1.82–17.1)
Pratt *et al.* 1993	8/13 (62 %)	12/42 (29 %)	10.0 (2.20–46.5)
Singh *et al.* 1995	28/87 (32 %)	49/301 (16 %)	2.44 (1.42–4.20)
Iijama *et al.* 1997	13/125 (10 %)	52/951 (5 %)	2.01 (1.06–3.80)
Kim *et al.* 1998	4/10 (40 %)	4/35 (11 %)	5.17 (2.72–26.54
Muller *et al.* 1999	4/12 (33 %)	8/42 (19 %)	2.13 (0.51–8.87)
Rushworth *et al.* 2000	10/24 (42 %)	30/77 (39 %)	1.12 (0.44–2.84)
Poppe *et al.* 2003b	9/17 (53 %)	20/87 (23 %)	3.77 (1.34–10.63)
Total	104/456 (23%)	336/2957 (11%)	2.30 (1.80-2.95)

OR = odds ratio, CI = confidence interval

A meta-analysis of subfertile women undergoing IVF, included 4 prospective studies on 1098 women, showed that anti-thyroid antibodies is associated with a significantly higher rate of miscarriage (RR 1.99, 95% CI 1.42-2.79, P<0.001) (Toulis *et al.,* 2010).

Data concerning effect of anti-thyroid antibodies on preterm delivery rate is not consistent. A study involving 1179 Japanese women, (Iljima *et al.,* 1997) showed that women who were anti-TPO or anti-TG positive had similar preterm delivery rates to those women who were anti-thyroid antibody negative (4%, 3.1% and 3% respectively) however birth weight to women who were anti-TPO antibody positive was significantly lower than those who were antibody negative (3021 vs 3110g, p<0.05). In contrast, a study conducted on 1500 euthyroid Pakistani women (Ghafoor *et al.,* 2006), showed that anti-TPO positivity is associated with a higher preterm delivery rate (26.8 vs 8.0%, p<0.01). A large population-based study of 10,990 women, found that anti-thyroid antibodies in the 1st and 2nd trimester was associated with preterm PROM suggesting

that these antibodies maybe a marker for an inflammatory process making women susceptible to this complication. However only 9,981 women from the 10,990 were euthyroid (Cleary-Goldman 2008). An uncontrolled case series showed that autoimmune thyroid disease is associated with preterm delivery (Stagnaro-Green 2009).

A recent meta-analysis examined the affect of anti-thyroid antibodies on the rate of miscarriage and preterm birth (Thangaratinam *et al.,* 2011). This meta-analysis included 30 articles with 31 studies (19 cohort and 12 case-control) involving 12,126 women examined the association between thyroid autoantibodies and miscarriage and 5 studies with 12 566 women evaluated the association with preterm birth. Analysis of the cohort studies showed a >3 fold increase in the OR of miscarriage in the antibody positive women (OR 3.90, 95% CI 2.48-6.12; p<0.001). In the case-control studies, the OR for miscarriage was 1.80 (95% CI 1.25-2.60; p=0.002). There was a significant doubling in the odds of preterm birth with the presence of thyroid autoantibodies (OR 2.07, 95% CI 1.17-3.68; P=0.01). The mean concentration of serum TSH was significantly higher in the antibody positive group with pregnancy loss than the antibody negative group (by 0.51 mIU/L, p=0.007). They concluded that the presence of maternal thyroid autoantibodies is strongly associated with miscarriage and preterm delivery (Thangaratinam *et al.,* 2011). Another meta-analysis published the same year (Negro 2011), selected 23,000 patients showed an association between thyroid autoimmunity and preterm delivery (OR 1.67, 95% CI 1.44-1.94, P<0.001). A recent study, also showed that euthyroid women positive for thyroid antibodies were at increased risk of spontaneous preterm birth < 37 weeks gestation (RR 1.7, 95% CI 1.1-2.8) (Karakosta *et al.,* 2012) however there was no increased risk of SGA < 10^{th} centile for GA, including and excluding the preterm births (RR 0.9 and 1.1 respectively).

A meta-analysis of 11 prospective cohort studies involving 35,467 women found the relative risk of preterm delivery compared to the reference group to be 1.41 (95% CI 1.08-1.84, p=0.011, Figure 1.4). However, on subgroup analysis anti-TPO antibodies was associated with a relative risk of 1.69 (95% CI 1.19-2.41, p=0.003) whereas anti-

TG was not associated with an increased risk of preterm delivery (RR 0.88, 95% CI 0.60-1.29, p=0.513) (He *et al.,* 2012).

There is very little evidence linking thyroid autoimmunity and PE. A Finish study found that women who are anti-TPO or anti-TG positive in the 1st trimester of pregnancy were not more likely to develop PE (1.9% vs 2.2% and 1.1% respectively) (Mannisto *et al.,* 2010) and similar results were found in a Greek study were euthyroid antibody positive women were in fact less likely to develop gestational hypertension and preeclampsia (RR 0.6, 95% CI 0.2-1.7) (Karakosta *et al.,* 2012).

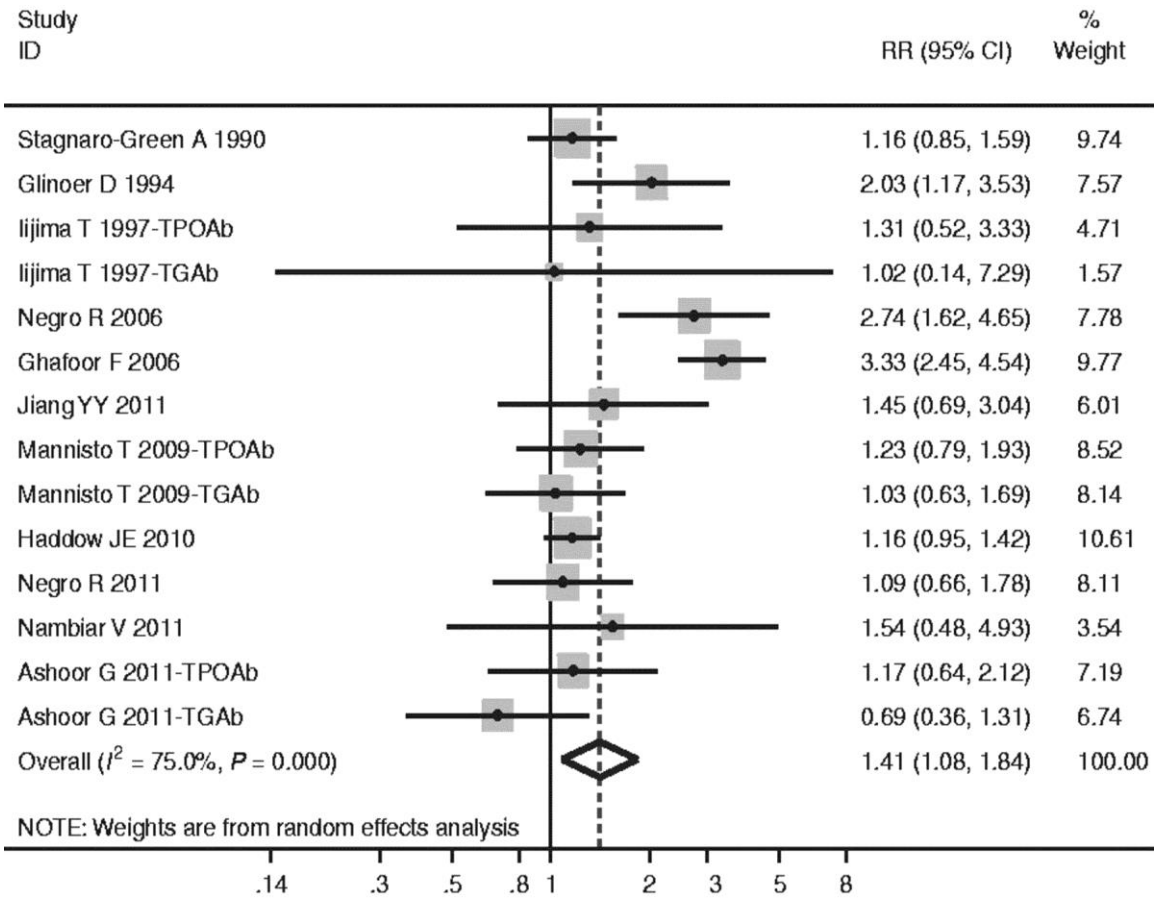

Figure 1.4. Forest plot showing the association between thyroid antibodies and risk of preterm delivery (He *et al.,* 2012).

A summary of 12 studies reporting on 966 thyroid antibody positive patients and 7331 controls, demonstrated an increased risk of subfertility (Figure 1.5) and miscarriage in patients with thyroid antibodies (OR 3.7, 95% CI 1.8-7.6; Figure 1.6). There was no evidence from 5 studies of an increased risk of miscarriage in IVF pregnancies (OR 1.6, 95% CI 0.76-3.5). However, patients with recurrent miscarriages more often had thyroid antibodies (OR 2.3, 95% CI 1.5-3.5, Figure 1.7) (van den Boogaard *et al.,* 2011).

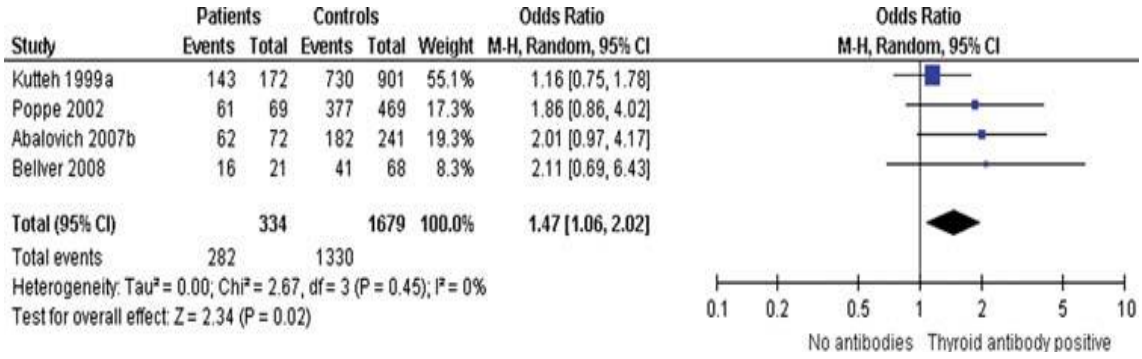

Figure 1.5. Forest plot of Odds Ratios and 95% Confidence Interval of pooled studies comparing euthyroid thyroid antibody positive patients with euthyroid antibody negative controls according to the risk of unexplained subfertility (van den Boogaard *et al.,* 2011).

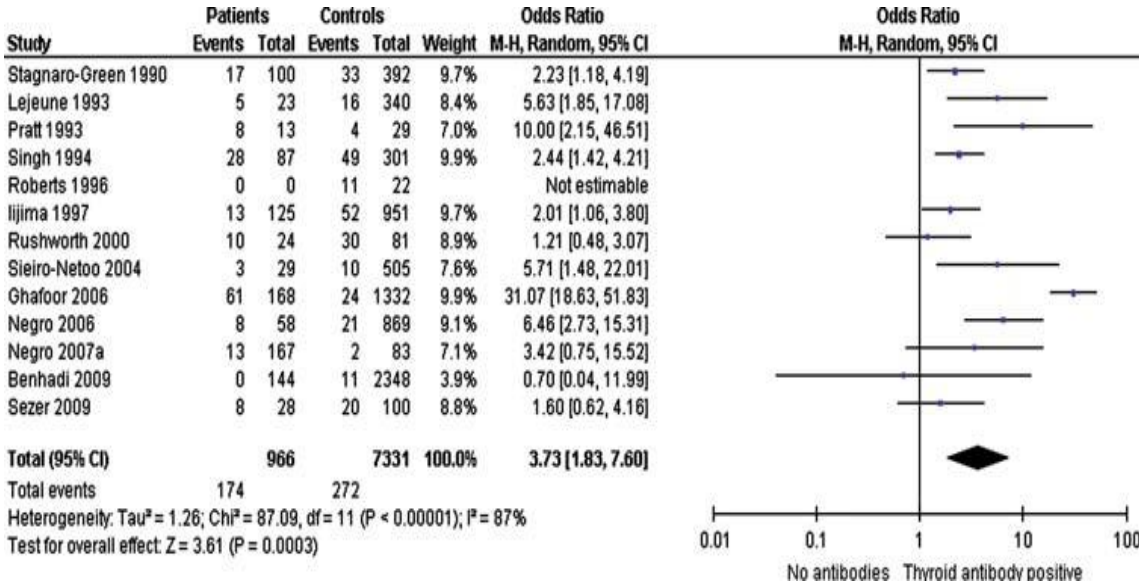

Figure 1.6. Forest plot of Odds Ratio's and 95% Confidence Interval of pooled studies comparing euthyroid thyroid antibody positive patients with euthyroid antibody negative controls according to the risk of miscarriage (van den Boogaard *et al.,* 2011).

There was no association with pregnancy-induced hypertension or PE (OR 1.4, 95% CI 0.42-4.8). This meta-analysis showed an increased risk of preterm delivery (OR 1.9, 95% CI 1.1-3.5; Figure 1.8), (van den Boogaard *et al.,* 2011).

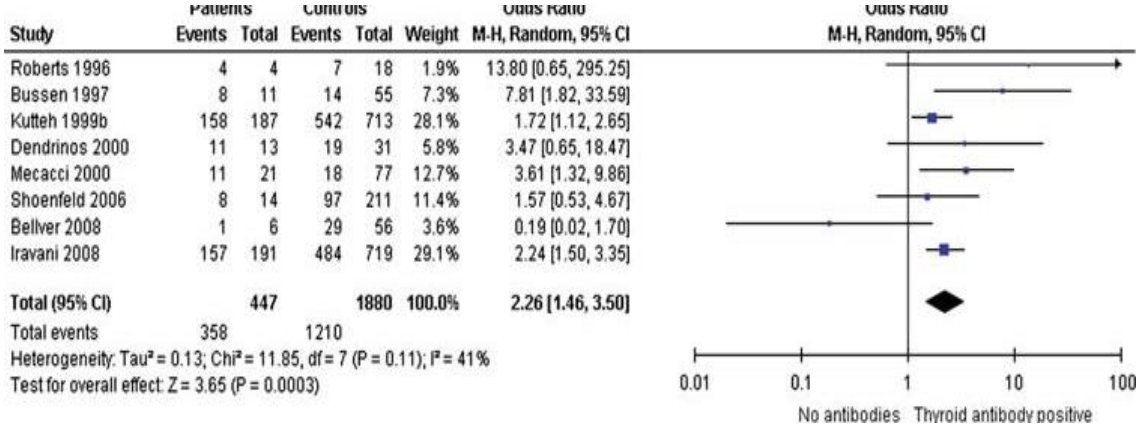

Figure 1.7. Forest plot of Odds Ratio's and 95% Confidence Interval of pooled studies comparing euthyroid thyroid antibody positive patients with euthyroid antibody negative controls according to the risk of recurrent miscarriage (van den Boogaard *et al.,* 2011).

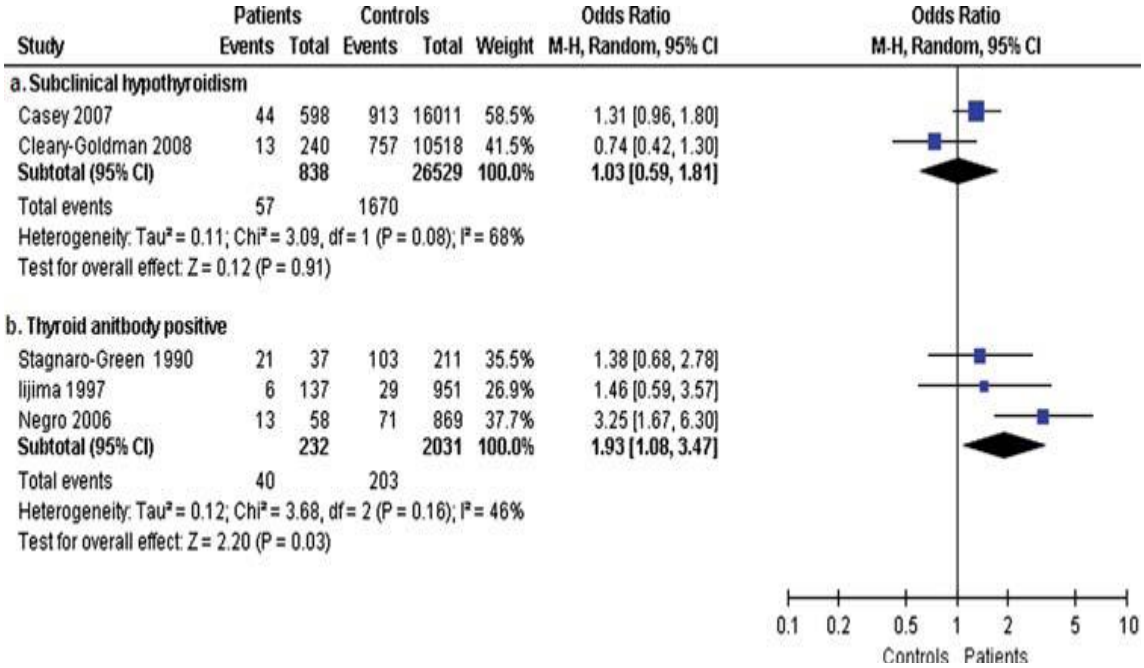

Figure 1.8. Forest plot of Odds Ratio's and 95% Confidence Interval of pooled studies comparing (a) patients with subclinical hypothyroidism with euthyroid controls and (b) euthyroid thyroid antibody positive patients with euthyroid antibody negative controls according to the risk of preterm delivery <37 weeks gestation.

Effects of treatment

In a prospective study of 1074 euthyroid women divided into 3 groups at the first antenatal visit, the anti-TPO positive given LT4, the untreated anti-TPO positive and the anti-TPO negative. This study confirmed that women who were anti-TPO positive had higher TSH (1.7 vs 1.1mIU/L; $p<0.05$), higher miscarriage rate (13.8 vs 2.4%; $p<0.01$) and higher prematurity rate (22.4 vs 8.2%; $p<0.01$) than the control group. The LT4 treated group compared to the untreated group had lower TSH and higher T4 levels, lower miscarriage rate (3.5% vs 13.8%; $p<0.05$) and lower premature delivery rate of <37 weeks gestation (7%vs 22.4%; $p<0.05$) (Negro *et al.,* 2006). It is accepted by the authors that the reduced miscarriage rate is not due to LT4 as most miscarriages occurred in the first trimester before treatment was started. The same group also showed that levothyroxine was not beneficial in a group of women undergoing assisted conception (Negro *et al.,* 2005). However, TSH was not measured in this study and therefore euthyroidism can not be confirmed. Autoimmunity in infertile women is possibly a larger factor in abnormal fertilization, implantation, and placental development, therefore LT_4 treatment alone may not be sufficient to improve outcomes. A meta-analysis of both these studies showed a significant reduction in miscarriages using levothyroxine by 52% (OR O.48, 95% CI 0.25-0.92,p=0.03) (Thangaratinam *et al.,* 2011). This group recommends the use of LT4 in women who have a TSH level > 2 mIU/liter and/or a high titer of anti-thyroid antibodies.

A retrospective study in a unit which routinely screens for thyroid dysfunction in pregnancy and treats women with 50 µg of LT4 if TSH >1 mU/L and anti-TPO is positive in the first-trimester showed that 49 women on treatment aiming for TSH level between 1-2 mU/L had a reduced rate of first-trimester miscarriage compared to the non-treated group (16.1% vs 0, p=0.02), however there was no difference in the rate of PE, preterm delivery or placental abruption. This study supports universal screening and treatment of autoimmune thyroid disease in pregnancy (Lepoutre *et al.,* 2012).

A prospective randomised placebo-controlled study on the use of selenium in anti-TPO positive women to prevent postpartum thyroiditis showed a reduction in the risk of

PPTD (28.6 vs.48.6%, p<0.01) on selenomethionine 200 µg/day starting after 12 weeks gestation. It seemed to have exerted an anti-inflammatory activity on the thyroid gland (Negro *et al.,* 2007b).

1.4 SUBCLINICAL HYPOTHYROIDISM

Subclinical hypothyroidism, defined as serum TSH above the upper limit of trimester specific reference range with normal serum FT4, is the first sign of mild thyroid dysfunction. Women who are anti-thyroid antibody positive are at increased risk of subclinical thyroid dysfunction. The prevalence of subclinical hypothyroidism in pregnancy is about 2.4% (Klein *et al.,* 1991; Glinoer *et al.,* 1995). Subclinical thyroid disease during pregnancy increases the risk of progression into overt thyroid disease 20 years later (Mannisto *et al.,* 2010).

The endocrine society clinical practice guideline (De Groot et al., 2012), recommends treatment of subclinical hypothyroidism who are anti-TPO positive as the potential benefits outweigh the potential risk. They state that the evidence for improved obstetric outcome is fair level B and for neurological outcome is poor level I. The panel also recommends treatment of subclinical hypothyroidism who are anti-TPO negative and the evidence for improved obstetric outcome is fair level C and for improved neurological outcome is poor level I (De Groot *et al.,* 2012).

1.4.1. Adverse pregnancy outcomes

The evidence for overt hypothyroidism causing adverse pregnancy outcomes led us to investigate whether subclinical hypothyroidism is also associated with these outcomes. Subclinical hypothyroidism was also shown to be associated with infertility and hyperprolactinaemia though the evidence is not consistent (Rodondi *et al.,* 2006; Gerhard *et al.,* 1991).

An early report of 12 pregnancies with subclinical hypothyroidism (TSH> 10 mIU/mL and T4 >4.5 µg/dL, nonpregnant range used) (Davis *et al.,* 1988), were at high risk of developing PE (17%, 2/12) and have birth weight <2500g (3/12, 2 were growth restricted and 1 was delivered preterm/diabetic). One case miscarried at 13 weeks, there was no spontaneous preterm labour (Davis *et al.,* 1988).

A prospective trial examining fetal loss in 4123 women who are anti-TPO antibody negative with either TSH levels between 2.5 and 5.0 in the 1st trimester or with TSH <2.5 and are not hyperthyroid (TSH undetectable and raised FT4) (Negro *et al.,* 2010b). Women with TSH between 2.5-5.0 had a higher fetal loss rate which included miscarriages and stillbirths (6.1 vs 3.6%, p=0.006). However there was no difference in the preterm delivery rate before 34 and 37 weeks between the 2 groups (Negro *et al.,* 2010b). Another study demonstrated the association between fetal loss and TSH levels in a cohort of 2497 women (Benhadi *et al.,* 2009). The incidence of fetal loss (miscarriages and stillbirths) increases 60% for every doubling in TSH levels (OR 1.6, 95% CI 1.04-2.47), this strong association was not seen with FT4 serum levels. Patients with overt disease were excluded from this study (TSH reference range used 0.34-5.60 mU/L and FT4 7.5-21.1 pmol/l) (Benhadi *et al.,* 2009).

Subclinical thyroid dysfunction and its association with PE was investigated after several case-control studies reported that in patients presenting with the clinical features of PE in the third trimester, thyroid function is disturbed with increase in maternal serum thyroid stimulating hormone (TSH) and decrease in the levels of thyroid hormones (Lao *et al*., 1988, 1990; Basbug *et al*., 1999; Khaliq *et al*., 1999; Larijani *et al*., 2004) or an increase in TSH and normal FT3/FT4 (Kumar *et al*., 2005). Some showed a correlation between TSH and severity of PE (Basbug *et al.,* 1999) and in severe cases although the total thyroid hormones levels are lower, the free fraction is higher than in controls (Larijani *et al.,* 2004).

A prospective study of 45 women with subclinical hypothyroidism (TSH> 5 mU/ml and FT4 between 4.5-13.2, normal range of nonpregnant population) showed an increased risk of PE and gestational hypertension, with 15% developing either (vs 7.6% in the

general population, p<0.01). They were also more likely to deliver infants <2500g (9 vs 6.8%). The increased prematurity rate was due to induction of labour for gestational hypertension (Leung *et al*., 1993). There was no increase in spontaneous preterm labour.

A large population based screening study of 9403 women where TSH was measured in the second trimester, defined thyroid deficiency as TSH >6 mU/l and if so the FT4 serum measurement was done. This group included both subclinical and overt hypothyroidism (the group with TSH 6-9.9 mU/l had a mean FT4 of 11.0 pmol/l and the group with TSH $\geq$10 mU/l had a mean FT4 of 9.6 pmol/l). The prevalence of TSH $\geq$6 mU/l was 2.2% and $\geq$ 10 mU/l was 0.4%. The rate of fetal death was significantly higher those with TSH $\geq$6 mU/l compared to those with TSH <6 mU/l (3.8% vs 0.9%, OR 4.4, 95% CI 1.9-9.5), these fetal deaths all occurred after 16-18 weeks. The prevalence of antithyroid antibodies however was higher in the thyroid deficiency group and so the association can not be attributed to either marker (9% vs 60%, p<0.001). However, the risk of the other adverse outcomes was not increased. There was no significant difference in mean gestational age at delivery, birth weight and pregnancy induced hypertension (Allan *et al.,* 2000).

In a large study of 17,298 patients who attended for prenatal care at or before 20 weeks gestation, were screened for thyroid dysfunction (Casey *et al.,* 2005) and 404 were found (2.3%) to have subclinical hypothyroidism (TSH>97.5th centile for gestational age and FT4 >0.680 ng/dL). They compared the pregnancy outcome of these women to those with TSH between the 5th and 95th centile. Placental abruption and preterm delivery (birth at or before 34 weeks) were increased in the women with subclinical hypothyroidism (RR 3.0, 95% CI 1.1-8.2 for abruption and RR 1.8, 95% CI 1.1-2.9 for preterm delivery), however risk of developing gestational hypertension or PE was not increased. The increased risk of prematurity was still present even when excluding women with TSH >10mU/L. The rate of neonates delivered <2500g was not increased in those with subclinical hypothyroidism, however the rate admission to intensive care and respiratory distress was twice as high (RR 1.8, 95% CI 1.1-2.9 and 1.0-3.3 respectively) both likely related to rate of prematurity. The rate of fetal death

was the same in both groups. The authors speculate that prematurity may be the link between

decreased neurodevelopment in women with subclinical hypothyroidism during pregnancy. In this study unlike Allan *et al.,* women who had low FT4 were excluded.

However, a screening study involving 10,990 women reported that subclinical hypothyroidism (TSH >97.5th centile and FT4 between 2.5th-97.5th centile for gestational age) in both the 1st and the 2nd trimester was not associated with increased risk for subsequent development of PE, miscarriage, preterm labour, preterm PROM and birth weight <2,500g (Cleary-Goldman *et al*., 2008). Another study found that in 5505 women with subclinical hypothyroidism (TSH >95TH centile corrected for gestation) in the 1st trimester of pregnancy were at significant risk of developing chronic hypertension and thyroid disease later on, however there was no significant increase in risk of PE (though risk was higher 3.8 vs 1.9%) (Mannisto *et al.,* 2010). Similar results were demonstrated in a study in Greece, women with subclinical hypothyroidism (TSH above reference range corrected for gestational age) were not at an increased risk of developing gestational hypertension and PE (RR 1.1, 95% CI 0.3-4.1) (Karakosta et al., 2012), spontaneous preterm birth (RR 0.3, 95% CI 0.1-2.4) or brith weight <10th centile for gestation age (RR 1.5, 95% CI 0.5-4.4).

Another recent population based study of 24,883 women, examined the rate of pregnancy hypertension (includes gestational hypertension, mild PE or severe PE) in women who were subclinically hyperthyroid, euthyroid and subclinically hypothyroid using the normal range of TSH as 0.03-4.13 mU/L (Wilson *et al.,* 2012). Patients with overt disease were excluded from the study (normal range FT4 0.9-2.0 ng/dL). The incidence of hypertension in pregnancy was 6.2%, 8.5% and 10.9% in the subclinical hyperthyroid, euthyroid and subclinical hypothyroid groups respectively (p=0.016). There was a significant association between subclinical hypothyroidism and severe PE (OR 1.6, 95% CI 1.1-2.4; p=0.03).

A meta-analysis was performed to establish whether subclinical thyroid function is significantly associated with adverse pregnancy outcome (van den Boogaard *et al.,*

2011). They showed an increases risk of unexplained subfertility (OR 4.0, 95% CI 1.7-9.8). There was no association with pregnancy-induced hypertension however there was a significantly increased risk of PE (OR 1.7, 95% CI 1.1-2.6). There was no association with preterm delivery (OR 1.0, 95% CI 0.59-1.8) (Figure x) or birth weight <2500g (OR 0.93, 95% CI 0.46-1.9).

Nerurodevelopmental delay

The only study on biochemically confirmed subclinical hypothyroid patients patients and neurodevelopmental delay was a done by group from China, they measured serum TSH, FT4 and anti-TPO antibodies in 1268 pregnant women at 16-20 weeks gestation (Li *et al.,* 2010). Patients with subclinical hypothyroidism (pregnancy specific reference ranges) had 8.88 lower mean intelligence scores (p=0.008) and 9.98 lower mean motor scores (p<0.001). Using a multivariate logistic regression analysis they found an independent effect of increased serum TSH and anti-TPO antibodies on intelligence (OR 15.63, OR 6.69, respectively) and motor scores (OR 8.25, OR 9.23, respectively).

1.4.2 Effect of treatment

The benefits of levothyroixne in women with subclinical thyroid dysfunction, has so far been examined only in infertile women, some undergoing ART (Velkeniers et al., 2013; Kim et al., 2011).

A randomised prospective case-control study of 64 infertile women with subclinical hypothyroidism biochemically and clinically, with TSH > 4.5 mIU/L and mean FT4 was 1.2 $\pm$ 0.2 SD were randomised to either LT4 treatment or no treatment (Kim *et al.,* 2011). The treatment group were given 50 µg of levothyroxine and they had a higher clinical pregnancy rate but did not reach statistical significance, the embryo implantation rate was also higher (p= 0.044), there were no miscarriages in the treatment group (0 vs 33.3%, p=0.021) and the live birth rate was significantly higher (53.1 vs 25 %, p=0.039).

The anti-thyroid antibody levels were similar between the pregnant and non pregnant groups in the treatment and control group, however the levels were higher in those that miscarried in the control group.

A meta-analysis of current evidence for subclinical hypothyroidism did not find sufficient evidence to advocate for levothyroxine treatment (Vissenberg et al., 2012) however another published meta-analysis of the benefits of levothyroxine in women with subclinical hypothyroidism undergoing ART (Velkeniers *et al.,* 2013), demonstrated a higher delivery rate (RR2.76, 95% CI 1.2-6.44, p=0.018), significantly lowered miscarriage rate (RR 0.45, 95% CI 0.24-0.82, p=0.01). There was no available data for its use for premature delivery and PE in the ART patients.

1.5 MATERNAL THYROID FUNCTION AND PREGNANCY COMPLICATIONS

1.5.1 Normal pregnancy

Reference ranges derived from non-pregnant individuals are inappropriate because pregnancy is associated with profound changes affecting thyroid function. Human chorionic gonadotrophin (hCG), whose levels increase during the first 10 weeks of pregnancy and subsequently decrease, has thyrotropic properties causing an increase in serum T4 and decrease in TSH (Yoshimura and Hershman, 1995). The concentration of thyroid binding globulins increases with gestation as a result of estrogen stimulation and therefore measurement of the total amount of thyroid hormones does not provide an accurate reflection of active free (F) fraction of these hormones (Ain *et al.,* 1987; Glinoer, 1997).

Several studies reported reference ranges of thyroid function in early pregnancy (Table 1.3). However, these studies examined a small number of patients, or the gestational range was wide, maternal history of thyroid disease was not recorded, anti-thyroid antibodies were either not measured or patients with such antibodies were not excluded, or they did not examine serum TSH with both FT3 and FT4 (Smith and Bold 1983; Chan and Swaminathan, 1988; Leylek *et al.,* 1996; Panesar *et al.,* 2001; Haddow

et al., 2004; Kurioka *et al.,* 2005; Dashe *et al.,* 2005; Stricker *et al.,* 2007; Casey *et al.,* 2007; Cotzias *et al.,* 2008; Lambert-Messerlian *et al.,* 2008; Gilbert *et al.,* 2008; McElduff *et al.,* 2008; Marwaha *et al.,* 2008; Pearce *et al.,* 2008).

1.5.2 Pregnancy in women with hypothyroidism treated with thyroxine

Pregnancy is associated with an approximate 50% increase in demand for thyroid hormones which is apparent within the first 16 weeks of gestation (Alexander *et al.,* 2004). This increase is mainly attributed to the estrogen-driven doubling in thyroxine-binding globulin concentrations (Glinoer *et al.,* 1990).

In women with pre-existing hypothyroidism treated with levothyroxine the increased demands for thyroid hormones in pregnancy should be met by increasing the dose of the drug (Alexander *et al.,* 2004; McDougall and Maclin, 1995). Nevertheless, several studies have documented that in the first-trimester of pregnancy 30-50% of such women may be inadequately treated during this critical period for fetal development when the fetal brain is entirely dependent on maternal thyroid hormones (Hallengren *et al.,* 2009; McClain *et al.,* 2008; Morreale de Escobar *et al.,* 2000; Contempre *et al.,* 1993; Burrow *et al.,* 1994).

The evidence for inadequate therapy is based on the biochemical finding of high serum thyroid stimulating hormone (TSH) in the presence of normal free thyroxine (FT4) (Alexander *et al.,* 2004). However, assessment of thyroid function by TSH and FT4 alone may be insufficient because it is free tri-iodothyronine (FT3) which is ultimately responsible for the control of both metabolic activity and regulation of TSH production (Fish *et al.,* 1987).

Under normal circumstances the daily relative production of T3 to T4 by the thyroid gland is about 1:9. While the whole source of circulating T4 is the thyroid gland, 80% of circulating T3 is derived from peripheral deiodination of T4 (Izumi and Larsen, 1977; Larsen, 1975; Bianco *et al.,* 2002). In cases of hypothyroidism treated by the

Table 1.3. Summary of previous studies reporting on thyroid function in pregnancy. The values are medians or means with reported or estimated 95% confidence intervals. a= anti-TPO positive excluded; b= anti-TPO and /or anti-Tg positive excluded.

Author	N	Gestation	Ethnic and/or racial origin	Assay	TSH (mIU/L)	FT4 (pmol/L)	FT3 (pmol/L)
Smith *et al,* 1983	56	4-11 wks	UK	Amersham International	4.9 (2.7-7.1)	15.2 (11.9-18.5)	
Chan *et al.,* 1988	25	1st trimester	China	Abbot Diagnostics	0.7 (0-1.6)	13.7 (8.6-18.8)	3.9 (2.2-5.6)
Leylek *et al.,* 1996	20	<13 wks	Turkey	Immulite 2000	2.4 (0.4-4.4)	32.3 (19.4-45.2)	3.4 (1.9-4.9)
Panesar *et al.,* 2001	55	11 wks	China	Chiron Diagnostics	0.8 (0.03-2.3)	16.2 (11.1-22.9)	4.0 (3-5.7)
Haddow *et al.,* 2004	1,005 a	8-13 wks	USA: Mainly White	Immulite 2000	0.94 (0-3.1)	-	-
Kurioka *et al.,* 2005	119	<14 wks	Japan	Roche Diagnostics	1.1 (0-3.0)	18.1 (12.9-23.2)	5.5 (4.0-7.1)
Dashe *et al.,* 2005	2,326	11-13 wks	USA: 84% Hispanic, 12% Black	Immulite 2000	0.8 (0.01-3.7)	-	-
Stricker *et al.,* 2007	575 b	7-12 wks	Switzerland	Abbot Diagnostics	0.95 (0.07-2.8)	13.9 (10.5-18.5)	4.7 (3.5-6.3)
Casey *et al.,* 2007	17,298	6-20 wks	USA: 86% Hispanic, 10% Black	Immulite 2000	- (0.08-3.0)	- (11.6-24.5)	
Cotzias *et al.,* 2008	307 at 6-40 wks	1st trimester	UK: 45% White, 36% South Asian	Bayer Diagnostics	- (0-5.5)	- (10.0-16.0)	- (3.0-7.0)
Gilbert *et al.,* 2008	1,817 b	9-13 wks	Australia	Abbot Diagnostics	0.7 (0.02-2.2)	13.5 (10.4-17.8)	4.4 (3.3-5.7)
Lambert-Messerlian *et al.,* 2008	8,351 b	11-13 wks	USA: Mainly White	Immulite 2000	1.0 (0-3.0)	14.2 (10.3-18.4)	
McElduff and Morris, 2008	218 a	10-14 wks	Australia	Immulite 2000	0.8 (0-1.8)	15.7 (10.4-21.0)	5.5 (3.0-8.1)
Marwaha *et al.,* 2008	107	1st trimester	India	Roche Diagnostics	2.1 (0.3-5.6)	14.5 (11.5-20.1)	4.4 (1.4-6.1)
Pearce *et al.,* 2008	585 a	5-13 wks	USA: 77% White, 10% Black	Bayer Diagnostics	1.1 (0.04-3.6)	-	-

exogenous administration of levothyroxine normalization of the serum levels of T4 would not adequately address the deficiency in the production of T3 by the thyroid gland.

Whatever the dose of levothyroxine the serum T4 to T3 ratio will always be elevated (Fish *et al.,* 1987; Hennemann *et al.,* 2004). In patients undergoing total thyroidectomy, the dose of levothyroxine needed to maintain serum T3 at its normal endogenous pre-thyroidectomy level results in elevated serum FT4 concentration (Jonklaas *et al.,* 2008). The symptoms of hypothyroidism in patients treated with levothyroxine are abolished only with a dose resulting in supernormal FT4 and subnormal TSH (Toft and Beckett, 2003; Saravanan *et al.,* 2002). These results may essentially indicate that levothyroxine treatment is successful only when there is normalization of FT3.

1.5.3 Thyroid function in pregnancies resulting in fetal death

Clinical hypothyroidism is associated with subfertility and in those women who conceive there is a high risk of miscarriage (60%) and fetal death (Abalovich *et al.,* 2002; Glinoer, 1997). A study examining the outcome of pregnancies in women with primary hypothyroidism reported that the rate of miscarriage in 16 cases with overt hypothyroidism was 60% whereas in 99 women made euthyroid with adequate thyroxine treatment there were no miscarriages (Abalovich *et al.,* 2002). In another study of 29 pregnant women with overt hypothyroidism the rate of fetal death during the second and third trimesters was nine times higher than in 552 euthyroid women (Sahu *et al.,* 2010).

In subclinical hypothyroidism, there is contradictory evidence as to whether the rate of fetal death is increased or not. A prospective study of 63 pregnant women with hypothyroidism on thyroxine replacement reported that the rate of fetal loss at 7-20 weeks was significantly higher in women with TSH above 4 mU/L than in those with TSH at or below 4 mU/L (26.3% vs 6.3%) (Hallengren *et al.,* 2009). Another study measured TSH in sera obtained from women at 15-18 weeks as part of routine

screening for neural tube and chromosomal defects and reported that the rate of fetal death between the time of enrolment and term was significantly higher in women with TSH at or above 6 mU/l than in those with TSH below 6 mU/L (3.8% vs 0.9%) (Allan *et al.,*2000).

Two other studies reported a lack of association between subclinical hypothyroidism and fetal death. Casey et al, assessed thyroid function in all pregnant women attending for routine care in their hospital at or before 20 weeks of gestation (Casey *et al.,* 2005). The rate of fetal death between enrolment and delivery was 0.5% in both those with TSH at or above the 97.5th percentile and FT4 within the normal range and in those with TSH values between the 5^{th} and 95^{th} percentiles. Cleary-Goldman et al, assessed thyroid function in women undergoing screening for chromosomal defects at 11-13 weeks (Cleary-Goldman *et al.,* 2008). There were no significant differences in the rates of miscarriage before 24 weeks and perinatal death between women with serum TSH and FT4 between the 2.5^{th} and 97.5^{th} percentiles and those TSH at or above the 97.5th percentile and FT4 in the normal range (0.4% vs 0.6% and 0% vs 0.3% respectively). Possible explanations for the contradictory results of the above studies are firstly, the small number of fetal losses in the cases with thyroid deficiency, with only a total of 16 such losses in the four studies (Hallengren *et al.,* 2009; Allan *et al.,* 2000; Casey *et al.,* 2005; Cleary-Goldman *et al.,* 2008) and secondly, the timing of thyroid screening in the late first trimester or the second trimester when the majority of pregnancy losses had already occurred.

There is also controversy as to whether any possible association between subclinical hypothyroidism and fetal death is the direct consequence of the metabolic derangement or it is mediated by the coexistence of antithyroid antibodies (Stagnaro-Green and Glinoer 2004; Negro *et al.,* 2007a; Negro *et al.,* 2006). Studies in the first-trimester reported that the risk of early miscarriage was 2-4 times higher in antithyroid antibody-positive compared to antibody-negative women (Stagnaro-Green and Glinoer 2004). Antithyroid antibodies may exert a direct adverse effect on the pregnancy, they may serve as a marker for other autoimmune conditions which cause fetal death, women with thyroid autoimmunity may be euthyroid before pregnancy but develop subclinical or overt hypothyroidism during the first-trimester or such women suffer

subfertility and become pregnant at an older age which in itself is associated with increased risk of fetal loss.

1.5.4 Thyroid function in pregnancies that develop preeclampsia

Preeclampsia (PE), which affects about 2% of pregnancies, is a major cause of maternal and perinatal morbidity and mortality (WHO, 2005; Lewis, 2007; CEMACH, 2008; Witlin *et al*., 2000; Irgens *et al*., 2001). Recent evidence suggests that PE can be divided into early-PE requiring delivery before 34 weeks and late-PE with the former being associated with a high incidence of fetal growth restriction, whereas in late-PE fetal growth is usually normal (Yu *et al*., 2008). The underlying mechanism for the development of PE is thought to be impaired trophoblastic invasion of the maternal spiral arteries and their conversion from narrow muscular vessels to wide non-muscular channels independent of maternal vasomotor control (Brosens *et al*., 1967; Khong *et al*., 1986; Pijnenborg, 1996). Indirect evidence for impaired placental perfusion in pregnancies destined to develop PE has been provided by Doppler studies of the uterine arteries which showed increased pulsatility index (PI) which is evident from 11-13 weeks of gestation and this increase is particularly marked for early-PE (Martin *et al*., 2001; Plasencia *et al*., 2007; Poon *et al.,* 2009). Effective first-trimester screening for both early-PE and late-PE is provided by a combination of maternal demographic characteristics and medical history, uterine artery PI and maternal mean arterial pressure (MAP) (Poon *et al.*,2009).

Several studies have reported than in patients presenting with the clinical features of PE, thyroid function is disturbed with increase in maternal serum thyroid stimulating hormone (TSH) and decrease in the levels of thyroid hormones (Lao *et al.,* 1988; Lao *et al.,* 1990; Khaliq *et al*., 1999; Basbug *et al*., 1999; Larijani *et al*., 2004; Kumar *et al*., 2005). The results of a population based study in which serum TSH was measured in women on average 20 years after their first pregnancy highlighted further the interrelation between hypothyroidism and PE (Levine *et al*., 2009b). Women who had PE in their first pregnancy were 1.7 times as likely to have increased serum TSH than women who had not had PE and those who had PE in both their first and second

pregnancies had a 5-7 fold increased likelihood for having high TSH. It was postulated that the effect of PE on thyroid function during and after pregnancy is mediated by the antiangiogenic factor soluble fms-like tyrosine kinase 1 (sFlt-1) which is elevated in patients with PE (Levine *et al*., 2009b). Patients with cancer treated with vascular endothelial growth factor inhibitors are at increased risk of developing hypothyroidism (Desai *et al*., 2006; Wolter *et al*., 2008; Feldman *et al*., 2009). Studies in mice have shown that the administration of sFlt-1 causes a major reduction in thyroid tissue capillary density and increased concentrations of TSH (Kamba and McDonald*,* 2007; Kamba *et al*., 2006). However, the suggestion that PE causes hypothyroidism is at least in part contradicted by the finding that women with hypothyroidism have an increased risk of developing PE (Echt and Doss, 1963; Davis *et al*., 1988; Leung *et al.,* 1993). Consequently, an alternative explanation for the findings of Levine et al, (Levine *et al*., 2009b) is that subclinical hypothyroidism may predispose to the development of PE, rather than the other way round. However, a first-trimester screening study reported that thyroid hypofunction in early pregnancy was not associated with increased risk for subsequent development of PE (Cleary-Goldman *et al.,* 2008).

1.5.5 Thyroid function in pregnancies delivering small for gestational age neonates

Small-for-gestational age (SGA) neonates, with birth weight below the 5th percentile, are at increased risk of perinatal death and handicap. The condition is heterogeneous and includes constitutionally small neonates and growth restricted ones due to impaired placentation, genetic disease or environmental damage. In normal pregnancy the spiral arteries in the placental bed are invaded by trophoblast, which becomes incorporated into the vessel wall and replaces the endothelium, muscular layer and neural tissue. These physiological changes convert the spiral arteries from narrow muscular vessels to wide non-muscular channels independent of maternal vasomotor control.

Impaired trophoblastic invasion and placentation is thought to be the underlying mechanism for many cases of preeclampsia (PE) and of impaired fetal growth in the absence of PE (Sheppard and Bonnar, 1981; Khong *et al.,* 1986; Pijnenborg *et al.,* 2006; Brosens *et al.,* 2011). Abnormal placentation is reflected in increased impedance to flow in the uterine arteries at 11-13 weeks gestation both in pregnancies that subsequently develop PE and to a lesser extent in those delivering SGA neonates in the absence of PE (Plasencia *et al.,* 2008; Karagiannis *et al.,* 2011).

The mechanism underlying trophoblast proliferation and invasion is largely unknown but there is some evidence implicating thyroid hormones in this process. In-vitro studies reported that thyroid hormones receptors are expressed in extravillous human trophoblast and thyroid hormones upregulate proliferation and the invasive potential of this trophoblast (Barber *et al.,* 2005; Oki *et al.,* 2004). Addition of thyroid hormones to an organ culture system of human placental tissue from early pregnancy stimulated the production of several placental hormones, including progesterone, human chorionic gonadotrophin and estradiol (Maruo *et al.,* 1991).

There is also contradictory evidence that clinical and subclinical hypothyroidism is associated with increased risk for both PE and the birth of SGA neonates in the absence of PE (Leung *et al.,* 1993; Levine *et al.,* 2009b; Sahu *et al.,* 2010; Blazer *et al.,* 2003; Casey *et al.,* 2007; Allan *et al.,* 2000). In a previous study, we reported that in pregnancies that develop PE maternal serum TSH at 11-13 weeks' gestation was higher and FT4 was lower than in normotensive controls (Chapter 6).

1.5.6 Thyroid function in pregnancies resulting in spontaneous preterm delivery

Preterm delivery is the main cause of neonatal death and neurological handicap in children (CMACE, 2010; Goldenberg et al., 2008; McCormick MC, 1985). Consequently, prediction and prevention of this complication is a major challenge in

pregnancy care. Whilst all births before 37 weeks' gestation are defined as preterm, the vast majority of mortality and morbidity relates to early delivery before 34 weeks.

A review of the literature concluded that both subclinical hypothyroidism and autoimmune thyroid disease in euthyroid women are associated with preterm delivery (Stagnaro-Green 2009). However, two large screening studies investigating the possible association between thyroid dysfunction and preterm delivery reported contradictory results. The first study measured serum TSH in 17,298 pregnancies attending for routine antenatal care before 20 weeks and reported that in the 404 women with subclinical hypothyroidism, compared to the euthyroid women, there was a 2-fold increase in risk of delivery before 34 weeks (4.5 vs 2.5%) (Casey *et al.* 2005). The authors speculated that prematurity may be the link between decreased neurodevelopment in the children of women with subclinical hypothyroidism during pregnancy (Haddow *et al.* 1999). In contrast, a second study assessed maternal thyroid function in 10,990 pregnancies at 10-13 weeks and reported that the rate of delivery before 37 weeks in 240 women with subclinical hypothyroidism was not significantly different than in euthyroid pregnancies (5.6 vs. 7.2%) (Cleary-Goldman *et al.* 2008).

These contradictory results may be the consequence of differences between the studies in the gestational age defining preterm delivery and the proportion of cases with iatrogenic rather than spontaneous preterm delivery which was not specified (Casey *et al.* 2005; Cleary-Goldman *et al.* 2008).

An additional factor that may account for contradictory results between studies is the distribution of maternal characteristics in the study populations. We have shown that in establishing references ranges for maternal thyroid function it is necessary to take into account certain maternal characteristics, which affect the measured levels of TSH and FT4 (Chapter 3). We have also found that in women with anti-thyroid antibodies, compared to the antibody negative group, the median TSH was higher and the median FT4 was lower and concluded that in establishing normal ranges of thyroid function it is necessary to exclude antibody-positive patients.

1.5.7 Thyroid function in pregnancies with fetal aneuploidies

Human chorionic gonadotrophin (hCG), which has an identical α-subunit and structurally similar ß-subunit to those of TSH, has thyrotropic properties and in early pregnancy there is an inverse association between maternal serum levels of TSH and hCG (Braunstein *et al.,* 1976. Yoshikawa *et al.,* 1989; Glinoer *et al.,* 1990; Ballabio *et al.,* 1991; Yoshimura and Hershman, 1995). Free ß-hCG is an established screening marker for trisomy 21 and we demonstrated that ß-hCG mRNA correlates with serum hCG levels (Banerjee *et al.,* 2005).

In pregnancies with fetal trisomy 21 the maternal serum concentration of free ß-hCG at 11-13 weeks' gestation is on average twice as high as in euploid pregnancies, whereas in trisomy 18 the levels are one fifth of normal (Macri *et al,* 1990; Spencer *et al.,* 1999; Tul *et al.,* 1999; Kagan *et al.,* 2008a).

It is therefore anticipated than in aneuploid pregnancies the maternal serum concentration of TSH would be altered. However, a case control study of 23 pregnancies with fetal trisomy 21 and 115 with euploid fetuses at 9-11 weeks reported that although in the unaffected pregnancies there was a correlation between serum hCG and TSH ($r=0.21$, $p=0.02$) there was no significant difference between the trisomic and euploid pregnancies in either hCG or TSH (Weinans *et al.,* 2001). This may be due to the early gestational age at measurement before the peak of serum hCG. In this study no corrections were made for maternal characteristics and gestational age that are known to affect the measured concentrations of hCG and TSH.

Maternal serum free ß-hCG decreases with gestational age and maternal weight, it is decreased in cigarette smokers and in parous women and it is increased in women of African racial origin and in those conceiving after ovulation induction drugs (Kagan *et al.,* 2008b).

1.6 OBJECTIVES OF THIS THESIS

The aims of this thesis are:

1. To establish reference ranges of serum TSH, FT3 and FT4 at 11-13 weeks' gestation in a large number of singleton pregnancies with no known thyroid disease and in the absence of anti-thyroperoxidase (anti-TPO) and anti-thyroglobulin (anti-Tg) antibodies and to examine the effect of maternal characteristics and serum anti-TPO, anti-Tg and free ß-hCG on the levels of TSH, FT3 and FT4.

2. To examine pregnant women with hypothyroidism treated by levothyroxine and investigate the interrelations between FT3, FT4 and TSH to offer a possible explanation for the finding of the coincidence of high serum TSH in the presence of normal FT4 in such patients.

3. To investigate the possible association between maternal thyroid dysfunction and fetal death by comparing serum TSH, FT4, FT3 and antithyroid antibody levels at 11-13 weeks' gestation in pregnancies ending in miscarriage or fetal death with those resulting in normal live births.

4. To investigate if the prevalence of maternal thyroid hypofunction at 11-13 weeks of gestation is higher in pregnancies that subsequently develop PE and if it is whether assessment of thyroid function can improve the prediction of PE provided by a combination of factors in the maternal history and the measurements of MAP and uterine artery PI.

5. To investigate if the prevalence of maternal thyroid hypofunction at 11-13 weeks' of gestation is higher in pregnancies that subsequently delivered SGA neonates in the absence of PE.

6. To estimate the possible association between maternal thyroid dysfunction and preterm delivery by comparing anti-thyroid antibody positivity and serum TSH and FT4 levels at 11-13 weeks' gestation, after appropriate adjustments for maternal

characteristics, in pregnancies which subsequently resulted in spontaneous delivery before 34 weeks with normal pregnancies delivering after this gestation.

7. To examine the association between maternal serum levels of TSH and hCG in trisomy 21, trisomy 18 and euploid pregnancies at 11-13 weeks, assess any differences in FT4 and FT3 between the three groups and investigate the potential value of TSH in first-trimester screening for aneuploidies.

8. To establish a normal range of serum TSH and FT4 in dichorionic and monochorionic twins at 11-13 weeks' gestation and compare the values to singleton pregnancies.

Chapter 2 Patients and Methods

ABSTRACT

The study population for this thesis was derived from a prospective screening study for adverse obstetric outcomes in women attending for their routine first hospital visit in pregnancy at 11^{+0}-13^{+6} weeks' gestation.

The maternal serum concentrations of FT3, FT4, TSH, anti-TPO and anti-Tg were measured by immunoassay using direct, chemiluminometric technology.

Maternal thyroid function was examined in:

- *Singleton pregnancies with normal outcome*
- *Singleton pregnancies resulting in miscarriage or fetal death*
- *Singleton pregnancies resulting in preeclampsia*
- *Singleton pregnancies resulting in spontaneous early preterm delivery*
- *Singleton pregnancies resulting in delivery of small for gestational age neonates*
- *Singleton pregnancies with fetal aneuploidies*
- *Singleton pregnancies with maternal hypothyroidism treated with thyroxine*
- *Dichorionic and monochorionic twins*

Thyroid function in each pregnancy group was compared to that in singleton pregnancies with normal outcome.

2.1 STUDY POPULATION

The study population for this thesis was derived from a prospective screening study for adverse obstetric outcomes in women attending for their routine first hospital visit in pregnancy at 11^{+0}-13^{+6} weeks' gestation.

2.1.1 Screening protocol

In the hospital visit at 11^{+0}-13^{+6} weeks' gestation, the following steps are carried out. First, we record maternal characteristics and medical history, secondly, we measure the mean arterial pressure (MAP), thirdly, we perform an ultrasound scan and fourthly, we obtain maternal blood.

Maternal history and characteristics

Patients are asked to complete a questionnaire on maternal age, racial origin (Caucasian, Afro-Caribbean, South Asian, East Asian and mixed), method of conception (spontaneous or assisted conception requiring the use of ovulation drugs), cigarette smoking during pregnancy (yes or no), history of any medical condition including hypothyroidism or hyperthyroidism (yes or no), any medication including thyroxine or antithyroid drugs (yes or no) and obstetric history including parity (parous or nulliparous if no previous pregnancies at or after 24 weeks) and previous pregnancy with PE (yes or no).

The questionnaire is then reviewed by a doctor together with the patient and the maternal weight and height are measured and the body mass index (BMI) is calculated in Kg/m^2.

Mean arterial pressure

The MAP is measured by automated devices (3BTO-A2, Microlife, Taipei, Taiwan) (Poon *et al*., 2009c). The pressure is measured in both arms simultaneously and a

series of recordings are made at 1-minute intervals until variations between consecutive readings fall within 10 mmHg in systolic blood pressure and 6 mmHg in diastolic blood pressure in both arms. When this point of stability is reached we calculate the MAP of each arm as the average of the last two stable measurements and take the arm with the highest final MAP.

Ultrasound scan

An ultrasound scan is carried out transabdominally and in cases when adequate examination is not possible the scan is performed transvaginally. The objectives of the scan are: firstly, to confirm gestational age from the measurement of the fetal crown-rump length (CRL) [Robinson], secondly, to diagnose any major fetal abnormalities and thirdly, to measure fetal nuchal translucency thickness (NT) (Snijders *et al.,* 1998). Additionally, the uterine artery PI is measured (Poon *et al*., 2009a). Essentially, transabdominal ultrasound and colour flow mapping is used to identify each uterine artery, pulsed wave Doppler is performed to measure the PI in the left and right arteries and the one with the lowest PI is recorded.

In twin pregnancies gestational age is calculated from the measurement of the fetal CRL [13] of the bigger twin. Chorionicity is determined from the presence or absence of the lambda sign [14].

Maternal blood sampling

Maternal blood was collected for measurement of serum free ß-hCG and PAPP-A (DELFIA EXPRESS analyzer, PerkinElmer, Waltham, USA) as part of screening for chromosomal abnormalities by a combination of fetal NT and serum biochemistry (Kagan *et al*., 2008b).

Additional blood was collected for research and the separated plasma and serum are stored at -80^{0}C for subsequent biochemical analysis. Written informed consent is obtained from the women agreeing to participate in the study, which was approved by King's College Hospital Ethics Committee.

Pregnancy outcome

Data on pregnancy outcome were collected from the hospital maternity records or the general medical practitioners of the women.

2.1.2 Study groups

During the study period (March 2006 to December 2006) we screened 4,852 singleton pregnancies with a live fetus at 11^{+0}-13^{+6} weeks. This population was used for deriving normal ranges and identifying subgroups with pregnancy complications. In some of the pregnancy complication groups, we identified additional cases from our screened population between January 2007 and October 2008. In the selection of the number of patients screened, a pragmatic view was undertaken that this number would contain sufficient cases of each of the pregnancy complications investigated by the thesis to draw valid conclusions.

Normal outcome

The study group included 4,318 of the 4,852 singleton pregnancies examined between March and December 2006. The inclusion criteria were pregnancies with no history of thyroid disease, which did not develop preeclampsia and resulted in live birth after 34 weeks of phenotypically normal neonates with birth weight above the 5th centile (13).

In this group there were 726 (16.8%) pregnancies in which the concentration of one or both antithyroid antibodies was 60 U/mL or more. Normal ranges for TSH, FT3 and FT4 were derived from the study of the 3,592 pregnancies with no antithyroid antibodies.

Maternal hypothyroidism

Thyroid function at 11-13 weeks was examined in 164 singleton pregnancies from

women who reported that they had hypothyroidism before pregnancy and they were receiving treatement with thyroxine.

The 164 cases included 69 from the screening study of 4,852 pregnancies between March and December 2006 and an additional of 95 cases examined between January 2007 and October 2008.

Miscarriage or fetal death

Thyroid function at 11-13 weeks was examined in 202 singleton pregnancies that subsequently resulted in miscarriage or fetal death.

The 202 cases included 87 from the screening study of 4,852 pregnancies between March and December 2006 and an additional of 115 cases examined between January 2007 and October 2008.

Preterm delivery

Thyroid function at 11-13 weeks was examined in 102 singleton pregnancies with no history of thyroid disease, resulting in spontaneous preterm delivery before 34 weeks' gestation of phenotypically normal neonates. The 102 cases included 51 from the screening study of 4,852 pregnancies between March and December 2006 and an additional of 51 cases examined between January 2007 and October 2008.

Preeclampsia

Thyroid function at 11-13 weeks was examined in 102 singleton pregnancies with no history of thyroid disease, resulting in preeclampsia. These cases were part of the 4,852 pregnancies examined between March and December 2006

Small for gestational age

Thyroid function at 11-13 weeks was examined in 212 singleton pregnancies with no

history of thyroid disease, which did not develop PE and resulted in live birth of phenotypically normal neonates with birth weight below the 5th percentile for gestational age

The 212 cases included 197 from the screening study of 4,852 pregnancies between March and December 2006 and an additional of 15 cases examined between January 2007 and October 2008.

Aneuploidies

Thyroid function at 11-13 weeks was examined in 30 singleton pregnancies with fetal trisomy 21, 25 with fetal trisomy 18 and 2 with paternally derived triploidy. The diagnosis of aneuloidy was made by chorionic villus sampling after first-trimester screening between March and December 2006.

Twin pregnancies

The study group included 235 twin pregnancies with no history of thyroid disease, which did not develop pre-eclampsia and resulted in live birth at or after 33 weeks of phenotypically normal neonates with birth weight above the 5th centile. Additionally, we examined 19 cases that developed severe twin-twin-transfusion syndrome (TTTS) requiring endoscopic laser surgery [17]. The pregnancies were examined between March 2006 and March 2011.

2.2 SAMPLE ANALYSIS

The maternal serum concentrations of FT3, FT4, TSH, anti-TPO and anti-Tg were measured by immunoassay using direct, chemiluminometric technology (Siemens Advia Centaur assays, Siemens Healthcare Diagnostics Ltd, Surrey, UK).

Details of TSH assay

The Adiva Centaur TSH assay used is an immunometric or non-competitive third generation assay where the analyte is reacted with an excess of labelled antibody as illustrated below. It has an improved limit of detection (0.003 mIU/L) compared to the first (0.1 mIU/L) and second (0.01 mIU/L) generation assay due to the use of antibodies with greater affinity for TSH, this also means using as little sample volume as possible (100µL).

The assay used is a sandwich assay with anti-TSH antibodies bound to it on either side on different sites and hence it is called non-competitive, this is possible since TSH is a large polypeptide analyte relative to the FT4 and FT3 nonpeptide hormones.

$$Ab_1 + Ag + Ab_2^* \longrightarrow Ab_1AgAb_2^* + Ab_1 + Ab_2^*$$

Initial state → Final state

Ab_1 = capture antibody; Ab_2^* = labelled antibody; Ag = Analyte (TSH)

The assay components include the reagent which is an acridinium ester (AE) labelled monoclonal mouse anti-TSH antibody (Ab_2^*) and the fluorescein-labelled anti-TSH antibody (Ab_1) which bind to different sites of the TSH. Once these antibodies are added to sample, all the TSH molecules should attach to these antibodies on either side forming a 'sandwich'. Then to remove any unbound AE-labelled antibody a solid phase separation method is used, the sample is added to solid phase-bound anti-Fluorescein antibody which is attached to a magnetic bead and when the magnet is turned on the TSH molecules will then attach to the bottom of the tube. Then, aspiration of the liquid phase occurs followed by washing the solid phase with buffer twice to ensure completeness of separation and to reduce any non-specific binding. Then the magnet is turned off to re-suspend the magnetic particles, this is an exceptionally rapid and efficient separation method. Then using chemiluminescence of the AE label that produces a flash of intense light means you can measure low concentrations of TSH due to greater sensitivity and a wider working range. The assay

is demonstrated in figure 2.1. The signal detected is directly proportional to the concentration of TSH in the serum as shown in figure 2.2.

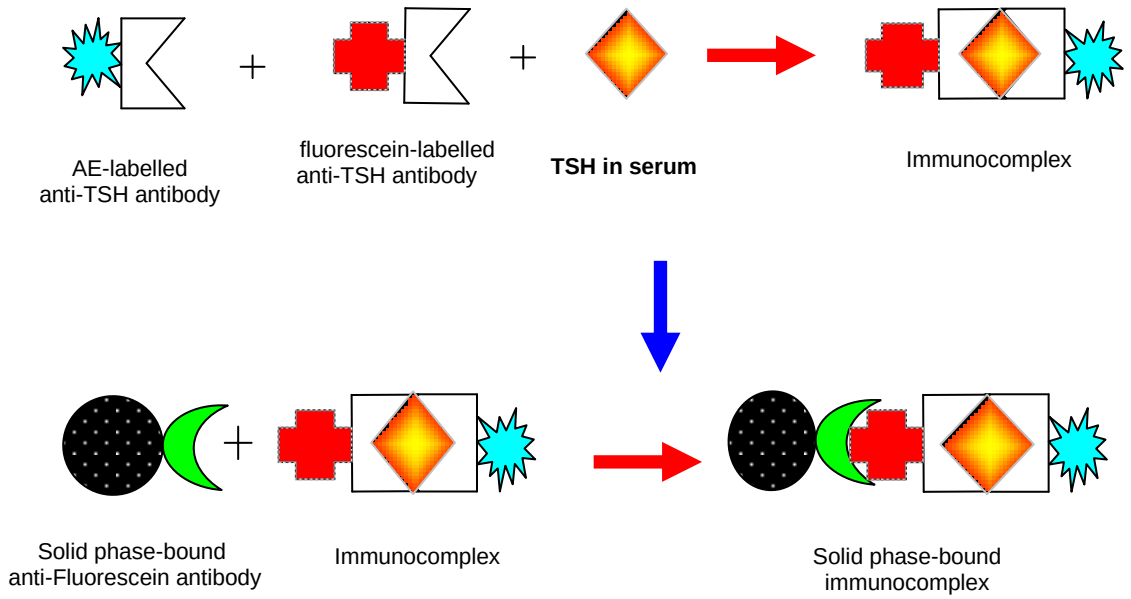

Figure 2.1. Constituents of the TSH Adiva Centaur assay. Taken from Siemens Healthcare Diagnostics, Frimley, Surrey UK.

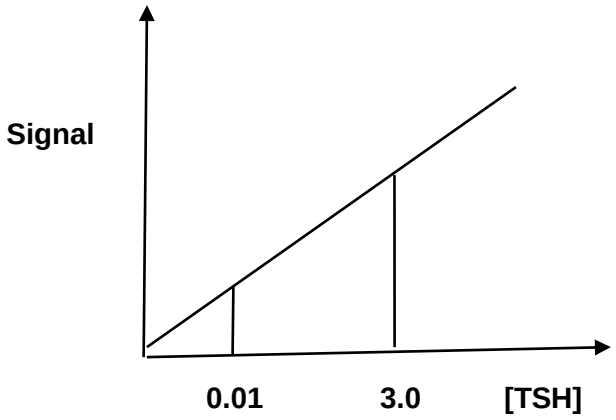

Figure 2.2. Graph showing chemiluminescence signal on y-axis and TSH concentration in mIU/L on x-axis.

Details of FT4 and FT3 assays

These free hormones are non-peptide hormones were measured by competitive immunoassay methods where the analyte in the sample competes with labelled analyte for the binding sites on a limited amount of antibody as illustrated below. The competition for binding sites must occur by ensuring that the amount of antibody is insufficient to bind all the labelled analyte. When equilibrium is reached, the amount of labelled analyte bound to the antibody will be inversely related to the amount unlabelled analyte in the sample. In determining the label in the bound fraction, this will provide a measure of the amount of analyte in the sample by using a calibration curve.

$$\underset{\text{Initial state}}{Ag + Ag^* + Ab} \longrightarrow \underset{\text{Final state}}{AgAb + Ag^*Ab + Ag^*}$$

Ag/Ag* = Free hormone/labelled free homone; Ab = antibody

The Advia Centaur FT4 and FT3 assays are one step analogue rapid, simple and high through put assays. There is minimal interference from autoantibodies such as the Rheumatoid factor and heterophilic antibodies and no serum protein interferences.

The FT4 Adiva Centaur assay is a competitive labelled analogue assay with a single incubation step (figure 2.3). The sample (25µL) is incubated with solid phase-coupled rabbit anti-T4 antibody (300µL) and AE-labelled T4 analogue (100µL). The AE-labelled T4 analogue is sufficiently similar to FT4 to bind the anti-T4 antibody, but is sufficiently dissimilar to prevent binding to serum binding proteins (figure 2.4). The solid phase separation method used is a paramagnetic particle attached to antibody and therefore a magnetic field and a buffer are used to remove excess labelled-T4.

Once an equilibrium is reached with both FT4 and labelled-T4 competing for the solid phase rabbit anti-T4 antibody, the excess unbound labelled-T4 is removed as described above. The higher the concentration of FT4 the less labelled-T4 is bound to

the solid phase rabbit anti-T4 antibody and therefore the less the signal. The graph plot between the signal and FT4 concentration has a sigmoid shape and therefore the assay is inaccurate at FT4 concentration <1.3 and >50 pmol/L as shown in figure 2.5.

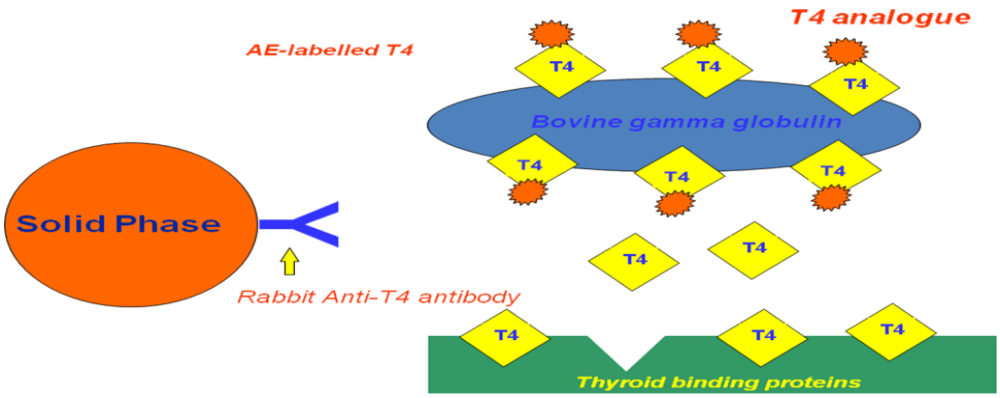

Figure 2.3. Constituents of the FT4 Adiva Centaur assay. Taken from Siemens Healthcare Diagnostics, Frimley, Surrey UK.

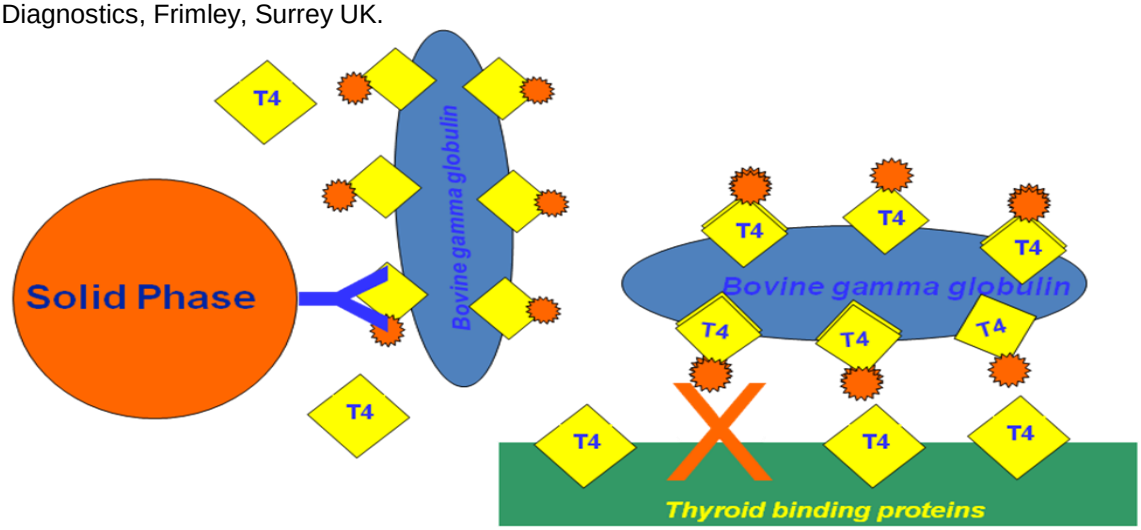

Figure 2.4. This figure demonstrates the competition for binding between the AE-labelled T4 analogue and the free serum T4. Taken from Siemens Healthcare Diagnostics, Frimley, Surrey UK.

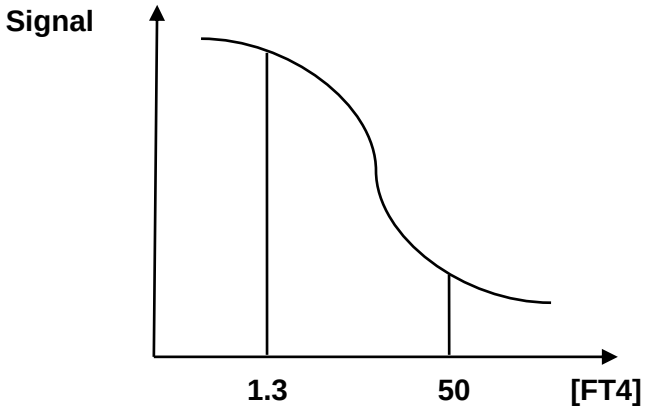

Figure 2.5. Graph showing chemiluminescence on y-axis and FT4 in pmol/L on x-axis.

The FT3 Adiva Centaur assay is also competitive assay with a single incubation step. The sample is incubated with abundant AE-labelled T3 antibody and the analogue diiodothyronine-bovine gamma globulin complex (T2-BGG). The serum FT3 and the analogue compete to bind to the AE-labelled antibody. The analogue is coupled to magnetic particles in solid-phase, the unbound AE-labelled T3 antibody will therefore bind to the analogue with magnetic particles and produce a signal (figure 2.6). This assay is also not adversely affected by the presence of abnormal concentrations of thyroid binding proteins.

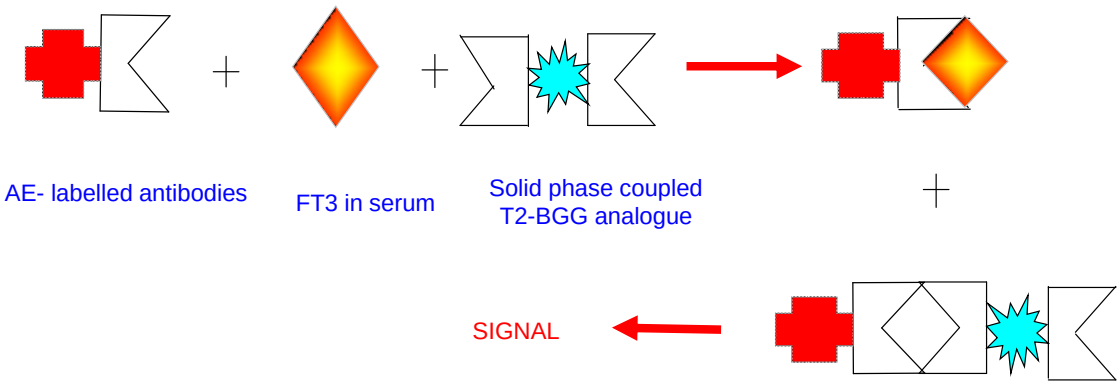

Figure 2.6. Constituents of the FT3 Adiva Centaur assay.

The higher the concentration of FT3 in the serum the lower the amount of analogue that binds to the labelled antibody and therefore the lower the signal produced. The graph is also sigmoid shaped similar to that of FT4, and therefore this assay is inaccurate at measuring FT3 levels <0.3 and >30 pmol/L as shown in figure 2.7.

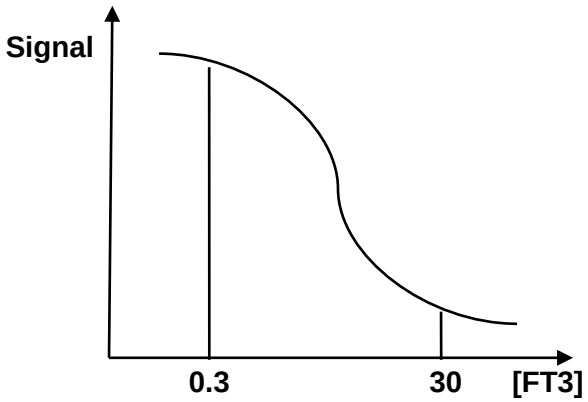

Figure 2.7. Graph showing chemiluminescence signal on y-axis and FT3 concentration in pmol/L on x-axis.

Details of Anti-TPO and anti-TG assays

The ADVIA Centaur anti-TPO assay is also a competitive immunoassay using chemiluminescent technology. Autoantibody against thyroid peroxidase in the patient sample competes with monoclonal mouse anti-TPO antibody covalently coupled to paramagnetic particles in the Solid Phase for a limited amount of human TPO complexed with acridinium ester-labeled monoclonal mouse anti-TPO antibody in the Lite Reagent (figure 2.8).

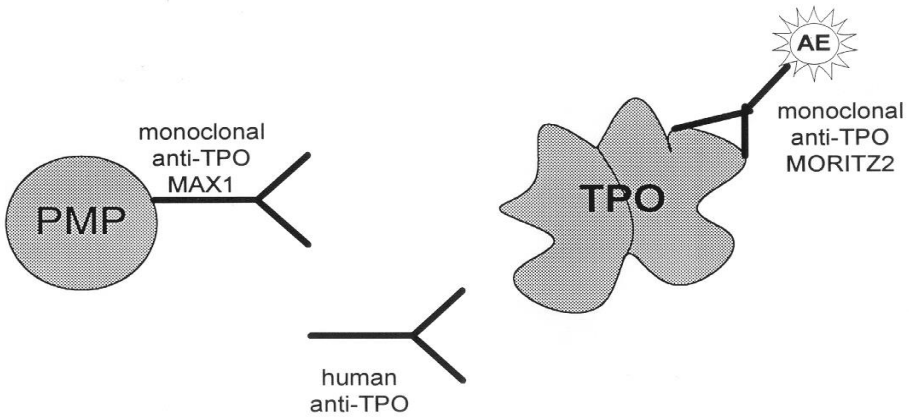

Figure 2.8. Competitive immunoassay for anti-TPO assay. Taken from Siemens Healthcare Diagnostics, Frimley, Surrey UK.

The ADVIA Centaur anti-Tg assay is a competitive immunoassay using direct, chemiluminescent technology. Autoantibody against thyroglobulin in the patient sample competes with polyclonal human anti-Tg antibody bound to polyclonal goat anti-human antibody covalently coupled to paramagnetic particles in the Solid Phase for a limited amount acridinium ester-labeled human thyroglobulin in the Lite Reagent as shown in figure 2.9.

In both assays there is an inverse relationship exists between the amount of anti-TPO and anti-Tg present in the patient sample and the amount of relative light units (RLUs) detected by the system. The cutoff used for positivity for both anti-TPO and anti-TG in our study was 60 U/ml (figure 2.10).

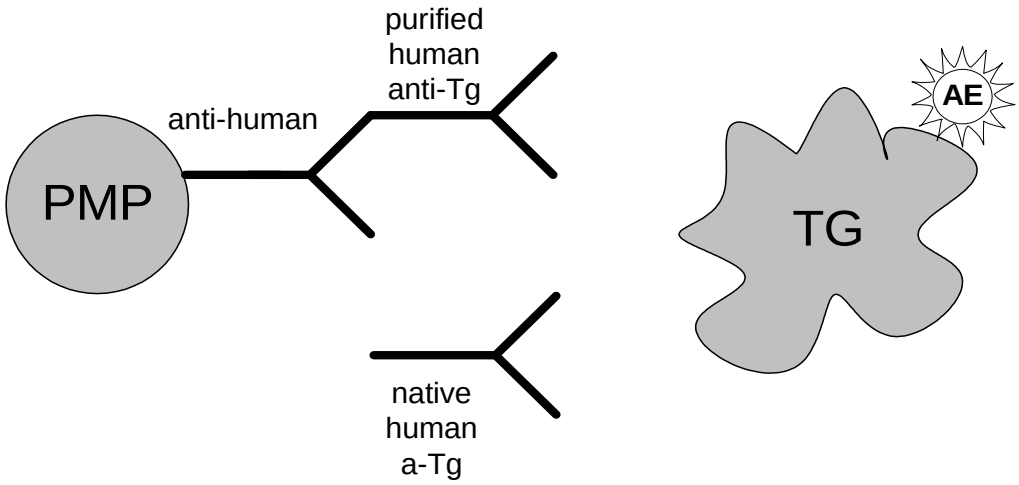

Figure 2.9. Competitive immunoassay for anti-Tg assay. Taken from Siemens Healthcare Diagnostics, Frimley, Surrey UK.

Figure 2.10. Graphs showing the inverse relationship between the signal in relative light units (y-axis) and level of anti-TPO antibody (left) and anti-TG antibody (right) on the the x-axis in U/ml. In our study 60 U/ml was the cutoff for positivity in both antibodies.

The minimum detectable concentrations of FT3, FT4, TSH, anti-TPO and anti-Tg are shown in Table 2.1. The intra-assay coefficients of variation (SD/mean x100) are summarised in Table 2.2. If the serum concentration of anti-TPO and anti-Tg was less than 60 U/mL, which was the manufacturer's reference limit, the patients were considered to be antibody negative.

Table 2.1. Minimum detectable concentrations of thyroid hormones and antibodies.

	Minimum detectable concentration
Free triiodothyronine (FT3)	0.3 pmol/L
Free Thyroxine (FT4)	1.3 pmol/L
Thyroid stimulating hormone (TSH)	0.003 mIU/L
Anti thyroid peroxidase antibody (anti-TPO)	15 U/mL
Anti thyroglobulin antibody (anti-Tg)	30 U/mL

Table 2.2. Intra-assay coefficient of variation of thyroid hormones and antibodies.

	Intra-assay coefficient of variation
Free triiodothyronine (FT3)	
Concentration 2.9 pmol/L	3.08%
Concentration 6.6 pmol/L	2.35%
Concentration 14.2 pmol/L	2.47%
Free Thyroxine (FT4)	
Concentration 6.1 pmol/L	4.69%
Concentration 13.9 pmol/L	2.31%
Concentration 39.9 pmol/L	2.22%
Thyroid stimulating hormone (TSH)	
Concentration 0.74 mIU/L	2.48%
Concentration 5.65 mIU/L	2.44%
Concentration 18.98 mIU/L	2.41%
Anti thyroid peroxidase antibody (anti-TPO)	
Concentration 1.70 U/mL	7.93%
Concentration 10.01 U/mL	4.54%
Concentration 14.95 U/mL	6.26%
Anti thyroglobulin antibody (anti-Tg)	
Concentration 62 U/mL	5.5%
Concentration 333 U/mL	2.9%

2.3 STATISTICAL ANALYSIS

The characteristics of the various study groups were compared by Mann Whitney test for continuous variables and Fisher's exact test or Chi-square test for categorical variables.

Intially TSH, FT3 and FT4 was checked for normality by the Kolmogorov-Smirnov test. This compares whether the scores in the sample set to a normally distributed set of scores with the same mean and standard deviation. If the test is not significant ($p>0.05$) then there is no significant difference from a normal distribution and therefore probably normally distributed. If there is a significant difference $p<0.05$, then it is not normally distributed. However in large samples size such as ours, a small deviation from normality can be statistically significant, but this deviation may not be significant enough to require a statistical procedure that we apply to the data, and therefore we plotted the data graphically as it is important to visually assess the extent of non-normality.

In the normal pregnancy group serum TSH, FT3 and FT4 were transformed to make their distribution Gaussian. Multiple regression analysis was then used to determine if gestational age at screening, maternal age, BMI, racial origin and method of conception were significant predictors of the transformed TSH, FT3 and FT4.

The measured values of TSH, FT3 and FT4 in each were expressed as multiples of the expected median (MoM) of normal. Comparison of TSH MoM, FT3 MoM and FT4 MoM between each pregnancy group was by Kruskal-Wallis test with post-hoc Bonferroni correction (critical statistical significance $p<0.0167$). The proportion of cases with serum TSH above the 97.5^{th} centile and serum FT3 and FT4 below the 2.5^{th} centile in each group were compared using the Chi-square test with post-hoc Bonferroni correction.

Regression analysis was used to determine the significance of the inter-relations between serum TSH, FT3 and FT4 and free ß-hCG.

Gaussian distribution.

The Gaussian distribution is a symmetric bell-shaped curve, a normal distribution where the mean, median and mode are the same.

Wilcoxon rank-sum test

The Wilcoxon rank-sum test is a nonparametric test used to compare 2 groups where the data is not normally distributed (test for normality is explained above), equivalent of the Student's t-test for normally distributed data.

This test ranks the data, so the lowest value is given a number 1, second lowest is 2 and so on, if there is no difference between the 2 groups then you would expect to find a similar number of low and high ranks in the 2 groups, and if you add up all the ranks then one would expect to find the summed total of ranks in each group to be the same. If they are different then the following calculation is made: the test statistic (W_s) is the lowest of these sums and to determine if the difference is significant, the mean ($\overline{W}_s$) and standard error ($SE_{\overline{W}s}$) is calculated:

$$\overline{W}_s = n_1(n_1 + n_2 + 1)/2$$

Where n1 is the sample size for group 1 and n2 is the sample size for group 2.

$$SE_{\overline{W}s} = \text{Sqrt } n_1 n_2 (n_1 + n_2 + 1)/12$$

Then we can convert these values into a z-score:

$$z = Ws - \overline{W}s / SE_{\overline{W}s}$$

If the z score >1.96 ignoring the minus sign then the test is significant at $p<0.05$ so there is a significant difference between the 2 groups.

Mann Whitney test

The Mann Whitney U test is the nonparametric equivalent of the Student's t-test as the t-test can be biased when the assumption of normality of distribution is not met. This test is calculated in a similar way to the Wilcoxon rank-sum test but uses a U score:

U= n1n2 + N1(N1+1)/2 – R1

Where n1 and n2 are the sample sizes, and R1 is the sum of ranks for group 1. The output will also give a p value to determine whether there is a significant difference.

Pearson's Chi-square test

The Chi-squared test is a nonparametric test to examine if there is a difference in proportions between two or more groups. It is used when the data compared is categorical and it approximates the p-value. The larger the sample size the better the approximation becomes. The Pearson's Chi-square (x^2) is calculated by:

$$\mathbf{x^2 = \Sigma\ (observed_{ij}\text{-}model_{ij})^2/model_{ij}}$$

where i represents the rows in the contingencey table and j represents the columns and $model_{ij}$ = expected frequency = row total x column total/total number of observations

The df is calculated as (r – 1)(c - 1) where r is number of rows and c is the number of columns and for each degree of freedom there is critical value at which $p<0.05$. If the observed value was bigger than the critical value then there was a significant relationship between the 2 variables

Fisher's exact test

The Fisher's exact test is a nonparametric test used also for catergorical variables giving an exact p-value. It is used when the sample size is small so using the Chi-

squared test is insufficient as it is an approximate p-value. It is often used when the expected frequency in each cell is <5 and therefore the sampling distribution is too deviant from a chi-square distribution, usually used on 2 x 2 contingency tables.

Kruskal-Wallis test

The Kruskal-Wallis test is the nonparametric equivalent of the one-way analysis of variance (ANOVA) when comparing more the two samples that are independent and not normally distributed. It is similar to the Mann-Whitney test where all the scores are ranked in order. The smallest values is given a rank 1, next highest is 2 and so on ignoring what group these scores belong to. Then all the scores are put back to their original groups and the ranks are summed up for each group and given a value R_i where *i* is used to denote the particular group. Then the statistic *H* is calculated by:

$$H = \frac{12}{N(N+1)} \sum \frac{R_i^2}{N_i} - 3(N+1)$$

Where N is the total sample size, n_i is the sample size for each particular group

The value of *H* has a special kind of distribution known as the Chi-squared distribution and the degree of freedom is one minus the number of groups.

The output of SPSS names *H* as Chi-squared due to its distribution, df (represents degrees of freedom) and the significance (p value). If Monte Carlo significance <0.05 then there is a statistically significant difference between the groups however it does not tell us exactly where the difference lies. To examine where the difference lies one needs to do multiple Mann-Whitney test however that will increase the Type 1 error and therefore a post-hoc Bonferroni correction is used.

post-hoc Bonferroni correction

This post-hoc Bonferroni correction is used for parametric and nonparametric data to adjust the significance level of the p-value when comparing more than 2 groups. In nonparametric data where multiple Mann-Whitney tests are made when there are > 2

groups, this would increase the type 1 error and therefore to correct for that so the type 1 error does not increase >0.05 we adjust the cut off for significance by the number of tests done. For example if 3 tests are made then the p values for significance is 0.05/3.

Regression analysis

Regression analysis is when we fit a model to our data and use it to predict values of the dependent vaiable (DV) from one or more independent variables (IVs). It is a way of predicting an outcome variable from one predictor variable (simple regression) or several predictor variables (multiple regression).

Linear regression analysis is a statistical model where one variable is dependent on the other and when plotted on Cartesian axes where x is the predictor variable and y is the dependent variable, a straight line forms. The strength of correlation is determined by Pearson correlation coefficient r.

Linear regression: $Yi = \beta_0 + \beta_1 xi + \varepsilon i$

The coefficients (the β_0 = y intercept of the line, β_1 = gradient of the straight line) and the noise terms ε1

The 'line of best fit' is the one with the least difference between the observed data points and the line also called the method of least squares (SS = sum of squares), however this does not assess the goodness to fit or how this model is a better predictor than 'our best guess'. R^2 represents the amount of variance the outcome variable can be explained by the model (SS_M) relative to how much variation there was to explain in the first place from the 'best guess' (SS_T)

$R^2 = SS_M / SS_T$

In simple regression, Pearson's correlation coefficient is the square root of R^2 and that gives us an overall fit of the regression model. The F-ratio represents how much the model has improved the prediction of the outcome compared to the level of inaccuracy of the model and is calculated by dividing the average sum of squares (also called

mean squares MS= SS/df) of the model (MS_M) by the residual mean squares (MS_R):

F-ratio = MS_M/MS_R

A good model would have a large F-ration at least >1.

The *t*-statistic tests the null hypothesis that the coefficient of a predictor variable is 0 and therefore the gradient of the regression line is also 0. The test tells us whether the *b*-value is different from 0 relative to the variation in *b*-value across samples.

$$t = b_{observed} - b_{expected}/SE_b$$

$b_{expected}$ here is 0, therefore

$$t = b_{observed}/SE_b$$

The df which is calculated as N-p-1 (N is total sample size, p is the number of predictors, linear regression df = N - 2) determines the distribution and significance of the t-statistic. If p<0.05 then *b* is significantly different from 0 and therefore the predictor makes a significant contribution to predicting the outcome.

Multiple regression analysis is a statistical model used when there are multiple predictor variables x1, x2,x3 and one dependent variable y.

Multiple regression: $Yi = \beta0 + \beta1\ (x1)i + \beta2\ (x2)i + \beta3\ (x3)i + \ldots + \beta K\ (xK)i + \varepsilon i$

The coefficients (the β's) and the noise terms ε1 ε2

Chapter 3 Thyroid function in normal pregnancy

ABSTRACT

Objective: To establish normal ranges of maternal serum thyroid stimulating hormone (TSH), free thyroxine (FT4) and free tri-iodothyronine (FT3) at 11-13 weeks of gestation.

Methods: Maternal serum concentrations of FT3, FT4, TSH, anti-thyroperoxidase (anti-TPO) and anti-thyroglobulin (anti-Tg) antibodies were measured at 11-13 weeks. Normal ranges were constructed from the data of singleton pregnancies with no antithyroid antibodies resulting in live birth after 34 weeks of phenotypically normal neonates with birth weight above the 5th centile. Adjustments were made for maternal characteristics found by multiple regression analysis to affect the levels of TSH, FT3 and FT4.

Results: 3,592 of the 4,318 pregnancies examined were antibody negative and in this group serum TSH increased whereas FT3 and FT4 decreased with gestation and all three were lower in Afro-Caribbean than in Caucasian women. Serum FT3 and FT4 decreased but TSH did not change significantly with maternal age, TSH and FT3 increased whereas FT4 decreased with body mass index, TSH decreased whereas FT3 and FT4 increased with serum free ß-hCG. In the antibody positive group, compared to the negative group, median TSH was higher and median FT3 and FT4 were lower.

Conclusion: The study established normal ranges for maternal thyroid function at 11-13 weeks.

This chapter is based on: Ashoor G, Kametas NA, Akolekar R, Guisado J and Nicolaides KH (2010) Maternal thyroid function at 11-13 weeks of gestation. *Fetal Diagn Ther,* 27:156-63.

3.1 INTRODUCTION

3.1.1 Background

Screening for thyroid disease in early pregnancy is hindered by the lack of appropriate reference ranges of thyroid function (see Chapter 1.5). Previous studies reporting reference ranges of thyroid function in early pregnancy examined a small number of patients, or the gestational range was wide, maternal history of thyroid disease was not recorded, anti-thyroid antibodies were either not measured or patients with such antibodies were not excluded, or they did not examine serum TSH with both FT3 and FT4 (see Chapter 1.5).

3.1.2 Objectives

The aims of this chapter are to establish reference ranges of serum TSH, FT3 and FT4 at 11-13 weeks' gestation in a large number of singleton pregnancies with no known thyroid disease and in the absence of anti-thyroperoxidase (anti-TPO) and anti-thyroglobulin (anti-Tg) antibodies and to examine the effect of maternal characteristics and serum anti-TPO, anti-Tg and free ß-hCG on the levels of TSH, FT3 and FT4.

3.2 PATIENTS AND METHODS

The study design and overall study population are described in Chapter 2.

In this study we retrospectively measured the maternal serum concentrations of FT3, FT4, TSH, anti-TPO and anti-Tg at 11-13 weeks in 4,318 pregnancies resulting in live birth of phenotypically normal neonates born after 34 weeks' gestation in the absence of preeclampsia and weighing above the 5th percentile for gestational age.

Sample analysis

The maternal serum concentrations of FT3, FT4 and TSH were measured by immunoassay as previously described in Chaper 2.

Statistical analysis

The characteristics of the antibody positive and negative groups were compared by Mann Whitney test for continuous variables and Fisher's exact test or Chi-square test for categorical variables.

In the antibody negative group, serum TSH, FT3 and FT4 were not normally distributed. After logarithmic transformation the distributions of FT3 and FT4 were Gaussian (Figure 3.1). However, $\log_{10}$ TSH remained negatively skewed therefore square root ($\sqrt{}$) transformation was applied (Figure 3.1). Multiple regression analysis was then used to determine if gestational age at screening, maternal age, BMI, racial origin and method of conception were significant predictors of $\sqrt{}$ TSH, $\log_{10}$ FT3, $\log_{10}$ FT4.

The observed values of TSH, FT3 and FT4 were expressed as multiples of the expected median (MoM) of normal. Regression analysis was also used to determine the significance of the inter-relations between serum TSH, FT3 and FT4 and free ß-hCG. Comparison of TSH MoM, FT3 MoM and FT4 MoM between the antibody positive and antibody negative groups was by Kruskal-Wallis test with post-hoc Bonferroni correction (critical statistical significance $p<0.0167$).

The proportion of cases with serum TSH above the 97.5th percentile and serum FT3 and FT4 below the 2.5th percentile in the antibody positive and negative groups were compared using the Chi-square test with post-hoc Bonferroni correction.

The statistical software package SPSS 16.0 (SPSS Inc.,Chicago, IL), Medcalc for windows, version 9.6.2.0 (MedCalc Software, Mariakerke, Belgium) and XLSTAT-Pro 2008 (Addinsoft, USA) were used for data analyses.

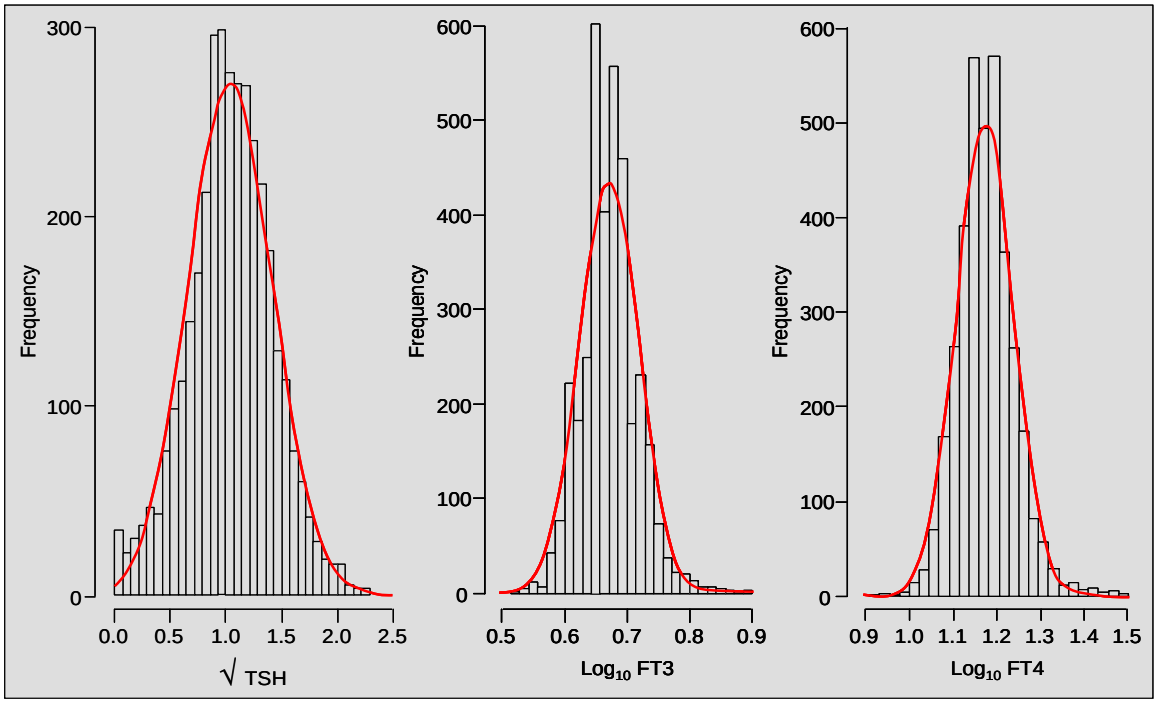

Figure 3.1. Frequency distribution of √ TSH, $\log_{10}$ FT3 and $\log_{10}$ FT4

3.3 RESULTS

In 3,592 of the 4,318 pregnancies examined the serum concentration of anti-TPO and anti-Tg was less than 60 U/mL and in 726 (16.8%) the concentration of one or both antibodies was 60 U/mL or more. The patient characteristics of the antibody negative and antibody positive groups are compared in Table 3.1. In the antibody positive group, the mean maternal age was increased and there was a higher proportion of Caucasian and South Asian women.

Reference range of serum FT3, FT4 and TSH

In the antibody negative group the distribution of √ TSH, $\log_{10}$ FT3 and $\log_{10}$ FT4 approximated a Gaussian normality (Figure 3.1).

Multiple regression analysis demonstrated that there were significant contributions to the level of TSH, FT3 and FT4 from maternal characteristics (Table 3.2). Serum TSH increased whereas FT3 and FT4 decreased with gestational age and all three were lower in Afro-Caribbean than in Caucasian women (Figure 3.2). Serum FT3 and FT4 decreased but TSH did not change significantly with maternal age. Serum TSH and FT3 increased whereas FT4 decreased with body mass index. The 50th, 95th, 97.5th, 5th and 2.5th percentiles of serum TSH, FT3 and FT4 are shown in Table 3.3.

Table 3.1. Comparison of maternal characteristics in the antibody negative and positive groups.

Maternal characteristics	Antibody negative n=3592	Antibody positive n=726
Maternal age in years, median (IQR)	32.2 (27.9-36.0)	33.2 (29.3-36.7)*
Body mass index in Kg/m^2, median (IQR)	24.7 (22.2-27.9)	24.5 (22.3 -28.2)
Crown-rump length in mm, median (IQR)	63.5 (59.0-68.7)	63.6 (58.9 -68.8)
Racial origin		
Caucasian, n (%)	2543 (70.8)	582 (80.2)*
Afro-Caribbean, n (%)	708 (19.7)	52 (7.0)*
South Asian, n (%)	148 (4.1)	63 (8.7)*
East Asian, n (%)	57 (1.6)	13 (1.8)
Mixed, n (%)	136 (3.8)	17 (2.3)
Conception		
Spontaneous, n (%)	3491 (97.2)	699 (96.3)
Ovulation drugs, n (%)	101 (2.8)	27 (3.7)

IQR=interquartile range

* Comparisons by Chi-square test with post-hoc Bonferroni correction for categorical variables (p<0.0167) and by Mann Whitney test for continuous variables (p<0.05).

There were significant associations between TSH MoM with FT4 MoM (*r*= -0.176, *p*<0.0001) and FT3 MoM (*r*= -0.107, *p*<0.0001) and between FT3 MoM and FT4 MoM (*r*=0.547, *p*<0.0001). There were significant associations between free ß-hCG MoM with TSH MoM (*r*= -0.156, *p*<0.0001), with FT3 MoM (*r*=0.135, *p*<0.0001) and with FT4 MoM (*r*=0.134, *p*<0.0001).

Table 3.2. Contribution of maternal and fetal characteristics to √ TSH, log_{10} FT4 and log_{10} FT3 demonstrated by multiple regression analysis.

Characteristics	Coefficient	Standard error	P value
Sqrt TSH			
Gestational age (weeks)	0.034864	0.012	0.0042
Body mass index (Kg/m^2)	0.002811	0.001	0.0002
Black racial origin	-0.182494	0.016	<0.0001
Asian racial origin	-0.133563	0.032	<0.0001
Oriental racial origin	-0.162253	0.050	0.0013
Log_{10} FT4			
Gestational age (weeks)	-0.006395	0.002	0.0026
Maternal age (years)	-0.000518	0.0002	0.005
Body mass index (Kg/m^2)	-0.001280	0.0002	<0.0001
Black racial origin	-0.010828	0.003	<0.0001
Asian racial origin	0.011146	0.004	<0.0001
Oriental racial origin	0.018198	0.009	0.038
Log_{10} FT3			
Gestational age (weeks)	-0.004332	0.002	0.032
Maternal age (years)	-0.001165	0.0001	<0.0001
Body mass index (Kg/m^2)	0.001138	0.0002	<0.0001
Black racial origin	-0.009081	0.002	<0.0001

Serum TSH, FT3 and FT4 in the antibody positive group

In 726 (16.8%) of the 4,318 pregnancies the concentration of one or both anti-TPO and anti-Tg was 60 U/mL or more. In 308 (7.1%) both antibodies were positive, in 133 (3.1%) only anti-TPO was positive and in 285 (6.6%) only anti-Tg was positive. The prevalence of antibody positivity was higher in Caucasian (582 of 3125, 18.6%) than Afro-Caribbean women (51 of 759, 6.7%; p<0.0001).

In the antibody positive group, compared to the antibody negative group, the median TSH was higher and the median FT3 and FT4 were lower (Table 3.4).

Table 3.3. Maternal serum concentration of TSH, FT4 and FT3 at 11-13 weeks' gestation.

Race	GA (wks)	BMI (Kg/m^2)	TSH (mIU/L)					Age (yrs)	FT4 (pmol/L)					FT3 (pmol/L)				
			2.5th	5th	50th	95th	97.5th		2.5th	5th	50th	95th	97.5th	2.5th	5th	50th	95th	97.5th
Caucasian	11	<25	0.08	0.16	1.05	2.69	3.09	<30	11.64	12.21	15.64	20.04	21.02	3.90	4.04	4.82	5.75	5.95
								>30	11.53	12.09	15.49	19.85	20.82	3.82	3.95	4.72	5.63	5.82
		>25	0.09	0.18	1.08	2.75	3.15	<30	11.44	11.99	15.37	19.69	20.65	3.96	4.10	4.89	5.84	6.04
								>30	11.33	11.88	15.22	19.51	20.46	3.88	4.01	4.79	5.72	5.92
	12	<25	0.10	0.19	1.12	2.81	3.22	<30	11.47	12.03	15.41	19.75	20.71	3.86	4.00	4.77	5.69	5.89
								>30	11.36	11.92	15.27	19.56	20.52	3.78	3.91	4.67	5.57	5.77
		>25	0.11	0.21	1.15	2.86	3.28	<30	11.27	11.82	15.14	19.41	20.35	3.92	4.06	4.85	5.78	5.98
								>30	11.16	11.71	15.00	19.22	20.16	3.84	3.97	4.74	5.66	5.86
	13	<25	0.13	0.23	1.19	2.93	3.34	<30	11.30	11.85	15.19	19.46	20.41	3.82	3.96	4.72	5.64	5.83
								>30	11.20	11.74	15.05	19.28	20.22	3.74	3.87	4.62	5.52	5.71
		>25	0.14	0.24	1.23	2.98	3.41	<30	11.11	11.65	14.92	19.12	20.05	3.88	4.02	4.80	5.73	5.92
								>30	11.00	11.54	14.78	18.94	19.86	3.80	3.93	4.70	5.60	5.80
Afro-Caribbean	11	<25	0.01	0.05	0.71	2.13	2.49	<30	11.36	11.91	15.26	19.55	20.50	3.82	3.95	4.72	5.63	5.83
								>30	11.25	11.79	15.11	19.37	20.31	3.74	3.87	4.62	5.51	5.70
		>25	0.01	0.06	0.73	2.18	2.54	<30	11.16	11.70	14.99	19.21	20.14	3.88	4.01	4.79	5.72	5.92
								>30	11.05	11.59	14.85	19.03	19.95	3.80	3.93	4.69	5.60	5.79
	12	<25	0.02	0.07	0.77	2.23	2.60	<30	11.19	11.73	15.04	19.27	20.20	3.78	3.91	4.67	5.58	5.77
								>30	11.08	11.62	14.89	19.08	20.01	3.70	3.83	4.57	5.46	5.65
		>25	0.02	0.08	0.80	2.28	2.65	<30	10.99	11.53	14.77	18.93	19.85	3.84	3.98	4.75	5.66	5.86
								>30	10.89	11.42	14.63	18.75	19.66	3.76	3.89	4.64	5.54	5.74
	13	<25	0.03	0.09	0.83	2.33	2.71	<30	11.03	11.56	14.82	18.98	19.91	3.75	3.87	4.62	5.52	5.71
								>30	10.92	11.45	14.67	18.80	19.72	3.67	3.79	4.53	5.40	5.59
		>25	0.04	0.10	0.86	2.39	2.77	<30	10.83	11.36	14.56	18.65	19.56	3.80	3.94	4.70	5.61	5.80
								>30	10.73	11.25	14.42	18.47	19.37	3.72	3.85	4.60	5.49	5.68

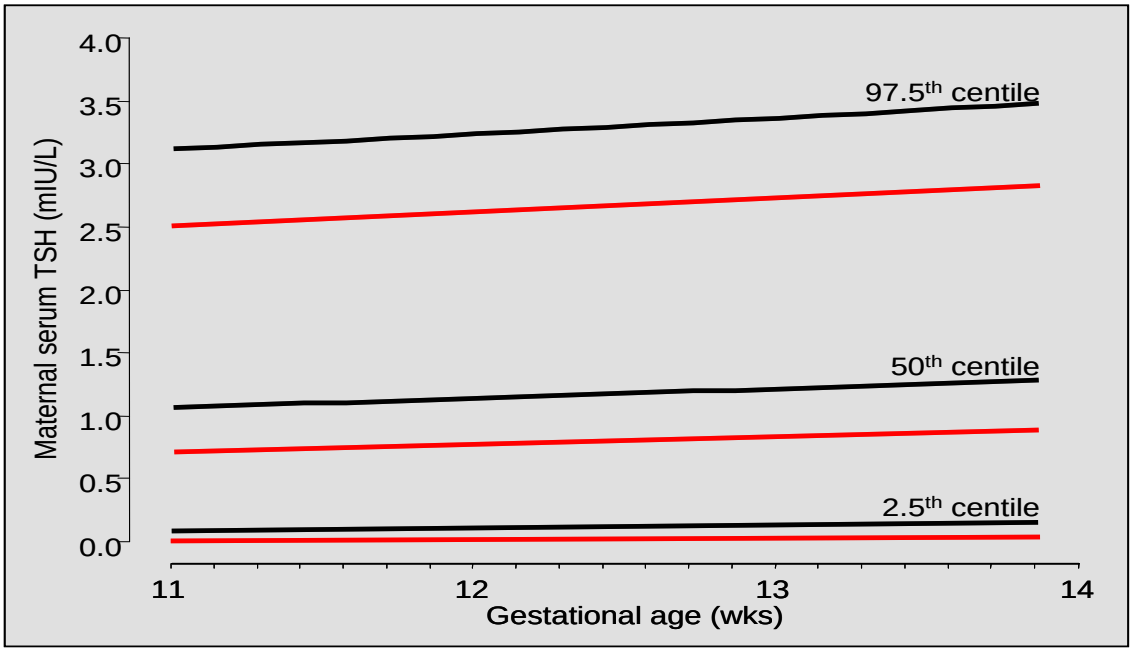

Figure 3.2. Reference range of maternal serum TSH with gestational age in Caucasian (black lines) and Afro-Caribbean (red lines) women.

Table 3.4. Comparison of the antibody positive and antibody negative groups for median TSH, FT3 and FT4 and proportion of cases with TSH above the 97.5th percentile of the reference range and FT3 and FT4 below the respective 2.5th.

Thyroid function	Antibody negative (n=3,592)	Antibody positive		
		Anti-TPO only (n=3)	Anti-Tg only (n=5)	Both (n=308)
Thyroid stimulating hormone				
Median MoM	1.01	1.53*	1.30*	1.80*
>97.5th centile, n (%)	89 (2.5%)	17 (12.8%)*	24 (8.4%)*	76 (24.7%)*
Free thyroxine				
Median MoM	0.99	0.98	1.01	0.96*
<2.5th centile, n (%)	89 (2.5%)	5 (3.8%)	9 (3.2%)	14 (4.5%)
Free triiodothyronine				
Median MoM	0.99	0.98*	0.98	0.97*
<2.5th centile, n (%)	89 (2.5%)	9 (6.8%)*	14 (4.9%)	17 (5.5%)*

*p<0.0167
Comparisons between each antibody group with no antibody group by Chi-square test with post-hoc Bonferroni correction for categorical variables and by Kruskal-Wallis with post-hoc Bonferroni correction for continuous variables.

Serum TSH was above the 97.5th percentile in 2.5% of the antibody negative group and increased to 5.3% (3 of 57) in the group with anti-TPO of 60-100 IU/mL, 15.1% (28 of 186) in the group with anti-TPO of 101-500 IU/mL and 31.3% (62 of 198) in the group with anti-TPO of more than 500 IU/mL. The respective values in the anti-Tg group were 10.4% (27 of 259), 21.1% (64 of 303) and 32.3% (10 of 31).

3.4 DISCUSSION

This chapter has established normal ranges of maternal serum TSH, FT3 and FT4 at 11-13 weeks' gestation. We excluded pregnancies complicated by miscarriage or fetal death, fetal growth restriction, preeclampsia and preterm delivery because of the reported association between these pregnancy complications and clinical or subclinical hypothyroidism (Leung *et al.,* 1993; Allan *et al.,* 2000; Casey *et al.,* 2005). We also excluded pregnancies with known thyroid disease and those with anti-thyroid antibodies.

We chose 11-13 weeks because this is the gestation at which pregnant women attend maternity units for their first antenatal visit. At this visit an ultrasound scan is carried out to determine the number of fetuses, confirm the gestation, exclude major defects and measure the fetal nuchal translucency thickness which in combination with maternal serum free ß-hCG and PAPP-A is used for effective screening of aneuploidies. Consequently, this is the likely gestation for screening for thyroid disease in pregnancy should such screening be accepted as a necessary part of routine antenatal care because it would be important to identify and treat hypothyroidism as early in pregnancy as possible.

Multiple regression analysis demonstrated that in our antithyroid antibody-negative normal pregnancies maternal characteristics and gestational age affect the serum concentrations of TSH, FT3 and FT4. Consequently, in establishing normal ranges we made adjustments for these factors by using the same multiple of the median approach as in the analysis of other metabolites, such as serum free ß-hCG. Previous

studies on maternal thyroid function in pregnancy have not made such adjustments and the observed differences in reported results may be a consequence of differences in maternal characteristics of the study populations, such as racial origin, age and body mass index. (Table 1.5; Smith *et al,* 1983; Chan *et al.,* 1988; Leylek *et al.,* 1996; Panesar *et al.,* 2001; Haddow *et al.,* 2004; Kurioka *et al.,* 2005; Dashe *et al.,* 2005; Stricker *et al.,* 2007; Casey *et al.,* 2007; Cotzias *et al.,* 2008; Gilbert *et al.,* 2008; Lambert-Messerlian *et al.,* 2008; McElduff and Morris, 2008; Marwaha *et al.,* 2008; Pearce *et al.,* 2008). Other possible factors contributing to the differences in results are the inclusion of patients with or without antithyroid antibodies, gestational age distribution of the pregnancies and reagents used for the assays.

Serum TSH increased and FT3 and FT4 decreased with gestational age within the narrow range of 11-13 weeks and this is likely to be the consequence of the thyrotropic properties of hCG whose concentration decreases with gestation. The finding that serum FT3 and FT4 decrease with maternal age, suggests that the function of the thyroid gland declines with age. A large study in non-pregnant individuals reported an age-related increase in both the mean serum TSH concentration and in the percentage

of people with high serum TSH concentration (>4.5 mIU/L) (Hollowell *et al.,* 2002). The finding that serum TSH increases and FT4 decreases with body mass index is compatible with the association between clinical and subclinical hypothyroidism with increased insulin resistance and the metabolic syndrome (Ruhla *et al.,* 2010; Verma *et al.,* 2008). We can not offer an explanation for the finding that FT3 increases with body mass index.

In women of Afro-Caribbean racial origin the serum concentration of both TSH and the thyroid hormones is lower than in Caucasian women. The results suggest that the pituitary-hypothalamus-thyroid gland axis in Afro-Caribbeans is set at a different level than in Caucasians but the underlying mechanism is uncertain. The finding of lower serum TSH in Afro-Caribbeans compared to Caucasians has also been reported in previous studies in both pregnant women and in non-pregnant individuals (Hollowell *et al.,* 2002; La'ulu and Roberts, 2007).

In about 10% of our population there were detectable anti-TPO antibodies and this prevalence is similar to the 5-15% rate reported in previous studies in the first trimester of pregnancy (Haddow *et al.,* 2004; Stricker *et al.,* 2007; Lambert-Messerlian *et al.,* 2008; McElduff *et al.,* 2008; Marwaha *et al.,* 2008; Pearce *et al.,* 2008). In 14% of our population there were anti-Tg antibodies and this prevalence is higher than the 3-9% rate reported in previous studies (Lambert-Messerlian *et al.,* 2008; McElduff *et al.,* 2008; Marwaha *et al.,* 2008).

The finding of lower antibody positivity in Afro-Caribbeans compared to Caucasians has also been reported in previous studies in both pregnant women and in non-pregnant individuals (Hollowell *et al.,* 2002; La'ulu and Roberts, 2007). In the antibody positive group, compared to the antibody negative group, the median TSH was higher and the median FT3 and FT4 were lower. This effect was observed for both anti-TPO and anti-Tg antibodies in contrast to a report in non-pregnant individuals that anti-Tg antibodies in the absence of anti-TPO does not affect thyroid function (Hollowell *et al.,* 2002).

In the antibody positive group the percentage of cases with TSH values above the 97.5th percentile increased with the serum antibody concentration. A previous study reported that the majority of antibody-positive women with subclinical hypothyroidism during pregnancy will develop clinical hypothyroidism within the subsequent 10 years (Haddow *et al.,* 1999). Consequently, in establishing normal ranges of thyroid function it is necessary to exclude antibody-positive patients.

3.5 CONCLUSIONS

The chapter established normal ranges for maternal thyroid function at 11-13 weeks' gestation after adjustment for maternal characteristics which affect the measured serum concentrations of TSH, FT3 and FT4. These ranges will form the basis for the study of thyroid function in pathological pregnancies and the investigation of the consequences of subclinical hypothyroidism.

Chapter 4 Thyroid function in pregnancies of women with hypothyroidism treated by thyroxine

ABSTRACT

Objective: The aim of this study in pregnant women with hypothyroidism treated by levothyroxine is to examine the interrelations between thyroid stimulating hormone (TSH), free thyroxine (FT4) and free tri-iodothyronine (FT3) and offer a possible explanation for the common finding of the coincidence of high serum TSH in the presence of normal FT4 in such patients.

Methods: This was a retrospective cross sectional study. Maternal serum concentrations of FT3, FT4 and TSH were measured at 11-13 weeks in 164 singleton pregnancies from women with hypothyroidism before pregnancy receiving treatement with thyroxine. The values were compared to the results in 4,318 normal singleton pregnancies.

Results: In the hypothyroid group, compared to the normal group, there was an increase in median TSH (1.990 vs 1.007 MoM) and FT4 (1.052 vs 0.992 MoM) and decrease in FT3 (0.901 vs 0.991 MoM). In both the hypothyroid and unaffected groups there were significant associations between TSH and FT4, TSH and FT3 and between FT3 and FT4. In 65 (39.6%) cases serum FT4 was above the 2.5th centile but either TSH was above the 97.5th centile and / or FT3 was below the 2.5th centile.

Conclusions: In a high proportion of pregnant women with hypothyroidism treated with levothyroxine there is evidence of persistent hypothyroidism because the treatment is inadequate in correcting the levels of FT3.

This chapter is based on: Ashoor G, Rotas M, Maiz N, Kametas NA and Nicolaides KH (2010) Maternal Thyroid Function at 11-13 Weeks of Gestation in Women with Hypothyroidism Treated by Thyroxine. *Fetal Diagn Ther,* 28:22-27.

4.1 INTRODUCTION

4.1.1 Background

Pregnancy is associated with an approximate 50% increase in demand for thyroid hormones which is mainly attributed to the estrogen-driven doubling in thyroxine-binding globulin concentrations (see Chapter 1.2). In women with pre-existing hypothyroidism treated with levothyroxine the increased demands for thyroid hormones in pregnancy should be met by increasing the dose of the drug, but several studies have documented that in the first-trimester of pregnancy 30-50% of such women may be inadequately treated (see Chapter 1.5). The evidence for inadequate therapy is based on the biochemical finding of high TSH in the presence of normal FT4, but assessment of thyroid function by TSH and FT4 alone may be insufficient because it is FT3 which is ultimately responsible for the control of both metabolic activity and regulation of TSH production (Fish *et al.,* 1987).

4.1.2 Objective

The aim of this chapter in pregnant women with hypothyroidism treated by levothyroxine is to examine the interrelations between FT3, FT4 and TSH and offer a possible explanation for the finding of the coincidence of high serum TSH in the presence of normal FT4 in such patients.

4.2 PATIENTS AND METHODS

The study design and overall study population are described in Chapter 2.

In this study we measured the maternal serum concentrations of FT3, FT4, TSH, anti-TPO and anti-Tg at 11-13 weeks in 164 singleton pregnancies from women who reported that they had hypothyroidism before pregnancy and they were receiving

treatement with thyroxine. The values were compared to the results of 4,318 normal singleton pregnancies with no history of thyroid disease, which did not develop PE and resulted in live birth after 34 weeks of phenotypically normal neonates with birth weight above the 5th centile (Chapter 3). The normal pregnancy group included 726 (16.8%) pregnancies in which the concentration of one or both antithyroid antibodies was 60 U/mL or more. Normal ranges for TSH, FT3 and FT4 were derived from the study of the 3,592 pregnancies with no antithyroid antibodies (Chapter 3).

Sample analysis

The maternal serum concentrations of FT3, FT4 and TSH were measured by immunoassay as previously described in Chaper 2.

Statistical analysis

The characteristics of the hypothyroid group with the group used for the construction of normal ranges were compared by Mann Whitney test for continuous variables and Fisher's exact test or Chi-square test for categorical variables. The measured concentrations of FT3, FT4 and TSH were converted to multiples of the expected normal median (MoM) corrected for gestational age and maternal age, racial origin and body mass index.

The hypothyroid and normal groups were compared for median TSH MoM, FT3 MoM and FT4 MoM using the Mann Whitney test and for the proportion of cases with serum TSH above the 97.5th percentile and serum FT3 and FT4 below the 2,5th percentile by the Chi-square test. In the hypothyroid group regression analysis was also used to determine the significance of the interrelations between TSH MoM, FT3 MoM and FT4 MoM.

The statistical software packages SPSS 15.0 (SPSS Inc.,Chicago, IL) was used for the data analyses.

4.3 RESULTS

The patient characteristics of the hypothyroid group with the normal pregnancy group (Chapter 3) are compared in Table 4.1. In the hypothyroid group, compared to the normal group, the maternal age was higher, there was a higher prevalence of Caucasian women and a higher prevalence of women who conceived after the use of ovulation induction drugs.

Table 4.1. Maternal demographic characteristics in the hypothyroid and normal groups.

Maternal variables	Normal (n=3,592)	Hypothyroid (n=164)
Maternal age in yrs (median, IQR)	32.2 (28.0-36.0)	35.4 (23.6-42.9)*
Body mass index in Kg/m^2, median (IQR)	24.7 (22.2-27.9)	24.9 (19.2-41.0)
Racial origin		
Caucasian, n (%)	2,543 (70.8)	134 (81.7)**
Afro-Caribbean, n (%)	708 (19.7)	12 (7.3)
Indian or Pakistani, n (%)	148 (4.1)	11 (6.7)
Chinese or Japanese, n (%)	57 (1.6)	4 (2.4)
Mixed, n (%)	136 (3.8)	3 (1.8)
Parity		
Nulliparous, n (%)	1684 (46.9)	64 (39.0)
Parous, n (%)	1908 (53.1)	100 (61.0)
Cigarette smoker, n (%)	322 (9.0)	10 (6.1)
Conception by ovulation drugs	101 (2.8)	11 (6.7)*

Comparison between hypothyorid and normal groups was by Chi square or Fisher exact test for categorical variables and Mann Whitney-U test for continuous variables. *p<0.001,

In the hypothyroid group, compared to the normal group, the median TSH MoM and FT4 MoM was increased whereas the median FT3 MoM was decreased (Table 4.2). The serum TSH was above the 97th percentile in 48 (29.3%) of the 164 cases, the FT3 was below the 2.5th percentile in 49 (29.9%) cases and the FT4 was below the 2.5th percentile in 6 (3.7%) cases (Figure 4.1). In all cases of low FT4, the serum TSH was above the 97.5th percentile. In 25 (52.1%) of the cases with low FT3, the serum TSH was above the 97.5th percentile.

On the basis of their serum TSH, FT4 and FT3 levels the 164 patients fell into one of five groups:

- 93 (56.7%) patients with serum TSH below the 97.5th percentile and both FT4 and FT3 above the 2.5th percentiles.
- 6 (3.7%) patients with TSH above the 97.5th percentile and both FT4 and FT3 below the 2.5th percentiles.
- 20 (12.2%) cases with TSH above the 97.5th percentile, FT3 below the 2.5th percentile and FT4 above the 2.5th percentile.
- 22 (13.4%) cases with TSH above the 97.5th percentile and both FT4 and FT3 above the 2.5th percentiles.
- 23 (14.0%) cases with FT3 below the 2.5th percentile, TSH below the 97.5th percentile and FT4 above the 2.5th percentile

Table 4.2. Maternal serum thyroid stimulating hormone, free thyroxine and free tri-iodothyronine values in the hypothyroid and normal groups

	Normal (n=3592)	Hypothyroid (n=164)
Thyroid stimulating hormone		
MoM (median, IQR)	1.007 (0.608-1.511)	1.990 (0.793-3.467)**
mIU/L (median, IQR)	1.096 (0.670-1.665)	2.435 (0.942-3.982)**
MoM >97.5 centile (%)	89 (2.5)	48 (29.3)**
MoM <2.5 centile (%)	89 (2.5)	5 (3.0)
Free thyroxine		
MoM (median, IQR)	0.992 (0.908-1.086)	1.052 (0.938-1.202)**
pmol/L (median, IQR)	14.9 (13.6-16.3)	15.8 (14.0-17.9)**
MoM >97.5 centile (%)	89 (2.5)	13 (7.9)**
MoM <2.5 centile (%)	89 (2.5)	6 (3.7)
Tri-iodothyronine		
MoM (median, IQR)	0.991 (0.935-1.059)	0.901 (0.818-0.957)**
pmol/L (median, IQR)	4.6 (4.4-5.0)	4.2 (3.8-4.5)**
MoM >97.5 centile (%)	89 (2.5)	4 (2.4)
MoM <2.5 centile (%)	89 (2.5)	49 (29.9)**

Comparison between each hypothyroid and normal groups was by Chi square or Fisher exact test for categorical variables and Mann Whitney-U test for continuous variables. ***p*<0.0001

In both the hypothyroid and normal groups, there were significant associations between TSH and FT4, TSH and FT3 and between FT3 and FT4 (Table 4.3, Figure 4.2).

Table 4.3. Correlations between serum thyroid stimulating hormone, free thyroxine and free tri-iodothyronine values in the hypothyroid and normal groups.

	Normal		Hypothyroid	
Correlations	**r**	**P**	**r**	**p**
TSH with FT3	-0.182	<0.0001	-0.550	<0.0001
TSH with FT4	-0.245	<0.0001	-0.474	<0.0001
FT3 with FT4	0.476	<0.0001	0.452	<0.0001

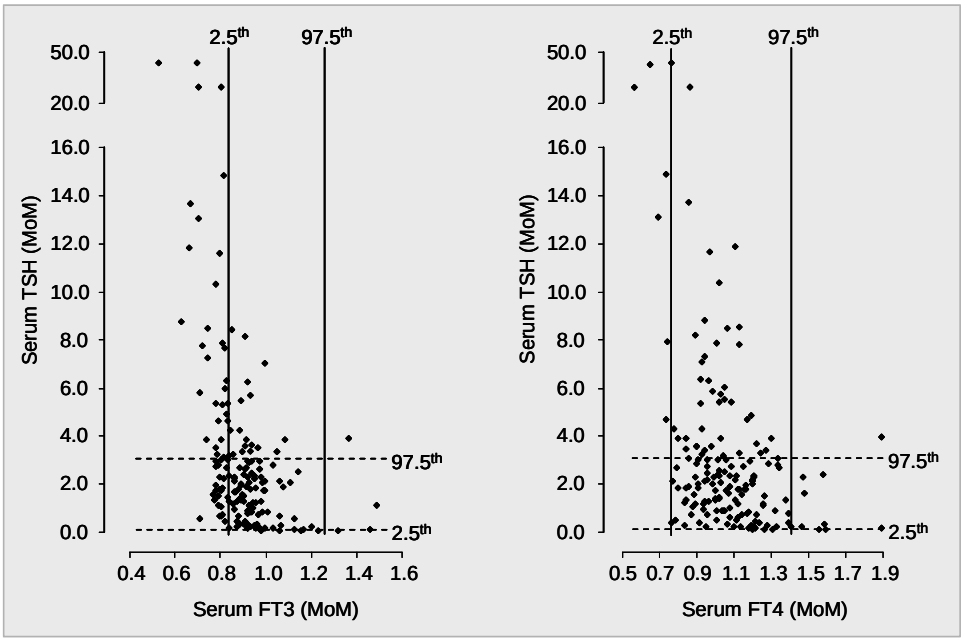

Figure 4.1. Relationship between maternal serum thyroid stimulating hormone (TSH) free tri-idothyronine (FT3) and free thyroxine (FT4) and in multiples of the expected normal median (MoM) at 11-13 weeks of gestation in pregnancies with pre-existing hypothyroidism treated with levothyroxine. The vertical lines represent the 2.5th and 97.5th percentiles of the normal ranges for FT3 and FT4 and the interrupted horizontal lines the 2.5th and 97.5th percentiles for TSH.

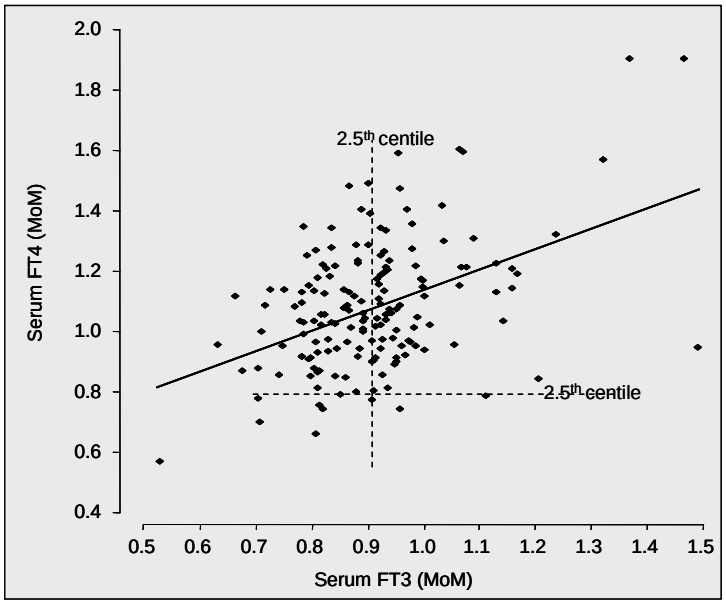

Figure 4.2. Relationship between maternal serum free tri-idothyronine (FT3) and free thyroxine (FT4) and in multiples of the expected normal median (MoM) at 11-13 weeks of gestation in pregnancies with pre-existing hypothyroidism treated with levothyroxine. The interrupted lines represent the 2.5th centiles of the normal ranges for FT3 and FT4.

Antithyroid antibodies

In normal group of 4,318 pregnancies, 726 (16.8%) were positive for one or both antithyroid antibodies (chapter 3). In this study of pregnancies with hypothyroidism treated with thyroxine the prevalence of antithyroid antibody positivity was increased to 73.2% (Table 4.4).

Table 4.4. Prevalence of antithyroid antibody positivity in the pregnancies with hypothyroidism treated with thyroxine in comparison with pregnancies with no known thyroid disease.

Pregnancy	n	Antibody positive			
		Anti-TPO	Anti-Tg	Both	Either
Hypothyroid	164	107 (65.2%)*	97 (59.1%)*	84 (51.2%)*	120 (73.2%)*
Unaffected	4318	441 (10.2%)	593 (13.7%)	308 (7.1%)	726 (16.8%)

* p<0.0001

4.4 DISCUSSION

This study has assessed thyroid function at the first obstetric visit at 11-13 weeks of gestation in women with known hypothyroidism diagnosed before pregnancy and receiving levothyroxine. In the hypothyroid group, compared to the unaffected group, there was a higher incidence of Caucasian women and the median maternal age was increased. These results are compatible with those of previous studies in non-pregnant individuals (Hueston and Pearson, 2004; Sawin *et al.,* 1985; Okamura *et al.,* 1989). The finding that in the hypothyroid group there was a higher incidence of women who conceived after the use of ovulation induction drugs is compatible with the knowledge that hypothyroidism is associated with impaired ovulation (Raber *et al.,* 2003; Joshi *et al.,* 1993). In the hypothyroid group the prevalence of antithyroid antibodies was substantially higher than in normal pregnancies. This is not surprising because in

developed countries autoimmune thyroiditis is the most common cause of hypothyroidism, especially in women of childbearing age (Hollowell *et al.,* 2002).

In the women with hypothyroidism treated with levothyroxine, there was a good inter-correlation between serum FT4, FT3 and TSH but the median FT4 and TSH were increased, whereas the median FT3 was decreased. On the basis of their individual results about 55% of the patients were biochemically euthyroid with normal serum TSH and normal or high FT4 and FT3. In the remaining 45% at least one of the three biochemical tests was suggestive of persistent hypothyroidism. There was a small group with low FT4 and FT3 and high TSH. In a much larger group, serum FT4 was normal or increased, but either TSH was high and / or FT3 was low. These findings raise the question as to whether the objective in the treatment of hypothyroidism in pregnancy should be to normalize TSH or FT4 or FT3.

In non-pregnant individuals with overt hypothyroidism, levothyroxine treatment is successful in abolishing their symptoms only with a dose resulting in supernormal FT4 and subnormal TSH (Toft and Beckett, 2003; Saravanan *et al.,* 2002). These results may essentially indicate that the treatment is only successful when there is normalization of FT3. Many of the symptoms of hypothyroidism, such as fatigue, constipation, weight gain, hair loss, dry skin and carpal tunnel syndrome, are common in normal pregnancy making it impossible to rely on such symptoms for monitoring success of treatment. In the management of pregnant women with hypothyroidism, it is recommended that the same approach should be used as in non-pregnant individuals where the objective of treatment is normalization of TSH (ACOG, 2002). However, such recommendation is not based on scientific evidence that in women with hypothyroidism treated with levothyroxine and normal serum FT4 pregnancy outcome is better in those with normal TSH than in those with high TSH. Indeed there is an inherent contradiction in the recommendation for the need to normalise TSH because the same professional body recommends against screening for subclinical hypothyroidism (high TSH with normal FT4) since there is no evidence that identification and treatment of women with this condition improves maternal or infant outcomes (ACOG, 2007).

Our study was a retrospective cross sectional one of patients examined in a university hospital clinic. We did not aim to examine the relation between our findings and the dose of levothyroxine, patient compliance, expertise of treating physicians or the interval between ingestion of the drug and blood sampling.

The findings provide a snapshot view of thyroid profile in early pregnancy in women with hypothyroidism treated with levothyroxine. We found that although the level of serum FT4 was invariably normal or increased in a high proportion of cases there was high TSH and low FT3, high TSH and normal FT3 or normal TSH and low FT3. Consequently, if the objective in the treatment of hypothyroidism in pregnancy is to normalize the levels of the biologically active FT3 it is not useful to monitor the levels of FT4 but it is essential to measure the levels of both TSH and FT3.

Recommendations on whether the objective in the treatment of hypothyroidism in pregnancy is to normalize TSH and / or FT3 rather than FT4 should ultimately be based on the results of major prospective studies examining the differential incidence of adverse pregnancy outcomes in the groups with low FT3 and normal TSH and FT4 and in those with high TSH and normal FT4 and FT3 compared to those in which all three biochemical markers are normal.

4.5 CONCLUSIONS

In a high proportion of pregnant women with hypothyroidism treated with levothyroxine there is evidence of persistent hypothyroidism because the treatment is inadequate in correcting the levels of FT3.

Chapter 5 Thyroid function in pregnancies resulting in fetal death

ABSTRACT

Background: Studies have shown that overt hypothyroidism is associated with a substantial risk of miscarriage. There is controversy as to whether subclinical hypothyroidism has the same effect and whether such effect is mediated by the presence of anti-thyroid antibodies. Our hypothesis is that maternal thyroid function in the first-trimester is altered in pregnancies ending in miscarriage or fetal death.

Methods: Thyroid stimulating hormone (TSH), free thyroxine (FT4), free triiodothyronine (FT3), anti-thyroperoxidase (anti-TPO) antibody and anti-thyroglobulin (anti-TG) antibody at 11-13 weeks of gestation were measured in 202 singleton pregnancies that subsequently resulted in miscarriage or fetal death and the values were compared to the results of 4,318 normal pregnancies.

Results: In the fetal loss group, compared to the normal group, there was an increase in median TSH multiple of the normal median (1.133 vs 1.007 MoM), decrease in median FT4 MoM (0.958 vs 0.992 MoM) and increase in the incidence of TSH above the 97.5th percentile 5.9% vs 2.5%) and FT4 below the 2.5th percentile (5.0% vs 2.5%). Logistic regression analysis demonstrated that in the prediction of fetal loss there were significant contributions from FT4 MoM, maternal Afro-Caribbean racial origin, history of chronic hypertension and use of ovulation drugs. The prevalence of antithyroid antibody positivity was not significantly different in the fetal loss group compared to that of normal pregnancies (15.3% vs 16.8%).

Conclusions: Impaired thyroid function may predispose to miscarriage and fetal death.

This chapter is based on: Ashoor G, Maiz N, Rotas M, Jawdat F and Nicolaides KH (2010) Maternal Thyroid Function at 11 to 13 Weeks of Gestation and Subsequent Fetal Death *Thyroid*, 20:989-93.

5.1 INTRODUCTION

5.1.1 Background

Clinical hypothyroidism is associated with a high risk of miscarriage and fetal death, but in subclinical hypothyroidism there is contradictory evidence as to whether the rate of fetal death is increased or not (see Chapter 1.5). There is also controversy as to whether any possible association between subclinical hypothyroidism and fetal death is the direct consequence of the metabolic derangement or it is mediated by the coexistence of antithyroid antibodies (see Chapter 1.5).

5.1.2 Objective

The aim of this chapter was to investigate further the possible association between maternal thyroid dysfunction and fetal death in the second and third trimesters by comparing serum TSH, FT4, FT3 and antithyroid antibody levels at 11-13 weeks' gestation in pregnancies ending in miscarriage or fetal death with those resulting in normal live births.

5.2 PATIENTS AND METHODS

The study design and overall study population are described in Chapter 2.

In this study we retrospectively measured the maternal serum concentrations of FT3, FT4, TSH, anti-TPO and anti-Tg at 11-13 weeks in 202 singleton pregnancies that subsequently resulted in miscarriage or fetal death (fetal loss group). The values were compared to the results of the normal outcome group of 4,318 singleton pregnancies with no history of thyroid disease, which did not develop pre-eclampsia and resulted in live birth after 34 weeks of phenotypically normal neonates with birth weight above the 5th centile (Chaper 3). The normal outcome group included 726 (16.8%) pregnancies in which the concentration of one or both antithyroid antibodies was 60 U/mL or more.

Sample analysis

The maternal serum concentrations of FT3, FT4 and TSH were measured by immunoassay as previously described in Chaper 2.

Statistical analysis

The characteristics of the fetal loss and unaffected groups were compared by Mann Whitney test for continuous variables and Fisher's exact test or Chi-square test for categorical variables. The measured concentrations of FT3, FT4 and TSH were converted to MoMs corrected for gestational age, maternal age, racial origin and body mass index (Chapter 3).

Comparison of TSH MoM, FT3 MoM and FT4 MoM between fetal loss and normal groups was by Mann Whitney-U test, with post-hoc Bonferroni correction (critical statistical significance $p<0.0167$). Logistic regression analysis was used to determine if maternal factors, TSH MoM and FT4 MoM had a significant contribution in predicting fetal loss. The performance of screening was determined by receiver operating characteristic (ROC) curves (Zweig and Campbell, 1993).

In the fetal loss and unaffected groups the Chi-square test was used to compare the proportion of cases with anti-TPO and anti-Tg antibodies and those with serum TSH above the 97.5th percentile and serum FT3 and FT4 below the 2.5th percentile.

The statistical software packages SPSS 15.0 (SPSS Inc.,Chicago, IL) was used for the data analyses.

5.3 RESULTS

The gestational age distribution at the time of miscarriage or the diagnosis of fetal death in the fetal loss group is shown in Figure 5.1. The patient characteristics of the fetal loss and unaffected groups are compared in Table 5.1. In the fetal loss group,

compared to the unaffected group, the median BMI was higher and there was a higher prevalence of Afro-Caribbean women, and women who conceived after receiving ovulation induction drugs.

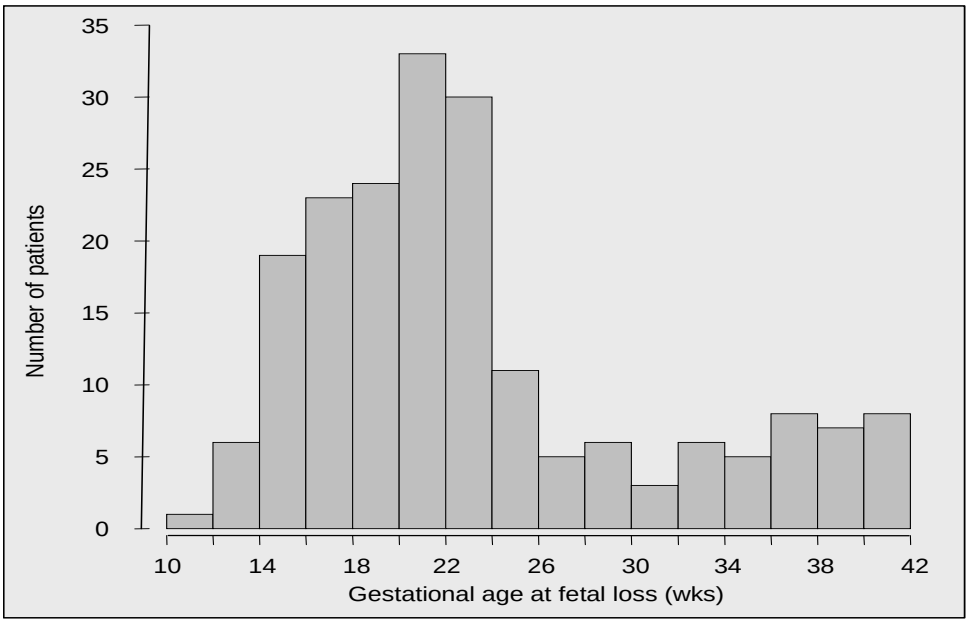

Figure 5.1. Gestational age distribution of miscarriage or fetal death

Table 5.1. Characteristics of the normal and fetal loss groups.

Maternal variables	Normal (n=3,592)	Fetal loss (n=202)
Gestation at sampling in wks (median, IQR)	12.4 (12.3-12.9)	12.4 (12.3-13.0)
Gestation at delivery in wks (median, IQR)	40.0 (39.0-40.9)	21.0 (17.7-25.7)**
Maternal age in yrs (median, IQR)	32.2 (28.0-36.0)	32.7 (26.1-36.8)
Body mass index in Kg/m^2, median (IQR)	24.7 (22.2-27.9)	26.9 (23.0-31.2)**
Racial origin		
White, n (%)	2,543 (70.8)	87 (43.1)
Black, n (%)	708 (19.7)	101 (50.0)**
Indian or Pakistani, n (%)	148 (4.1)	6 (3.0)
Chinese or Japanese, n (%)	57 (1.6)	1 (0.5)
Mixed, n (%)	136 (3.8)	7 (3.5)
Parity		
Nulliparous, n (%)	1684 (46.9)	97 (48.0)
Parous, n (%)	1908 (53.1))	105 (52.0)
Cigarette smoker, n (%)	322 (9.0)	17 (8.4)
Conception by ovulation drugs, n (%)	101 (2.8)	31 (15.3)**

Comparison between fetal loss and normal groups was by Chi square or Fisher exact test for categorical variables and Mann Whitney-U test for continuous variables. *p<0.05, ** p<0.0001

In the fetal loss group, compared to the normal group, the median TSH MoM was increased and the median FT3 MoM, and FT4 MoM were decreased (Table 5.2). Linear regression analysis in the fetal loss group showed that there was no significant association between the gestation at fetal loss and TSH MoM (p=0.654), FT3 MoM (p=0.411) and FT4 MoM (p=0.917). In the fetal loss group serum TSH was above the 97.5th percentile of the normal range in 12 (5.9%) cases and the serum FT4 was below the 2.5th percentile in 10 (5%) of cases. In 5 of the 10 cases with low FT4 serum TSH was high.

Table 5.2. Thyroid stimulating hormone, free thyroxine and free triiodothyronine values in the fetal loss and normal groups.

	Normal (n=3592)	**Fetal loss (n=202)**
Thyroid stimulating hormone		
MoM (median, IQR)	1.007 (0.608-1.511)	1.133 (0.639-1.621)*
mIU/L (median, IQR)	1.096 (0.670-1.665)	1.127 (0.638-1.714)
MoM >97.5 centile (%)	89 (2.5)	12 (5.9)*
Free thyroxine		
MoM (median, IQR)	0.992 (0.908-1.086)	0.958 (0.864-1.048)**
pmol/L (median, IQR)	14.9 (13.6-16.3)	14.0 (12.8-15.5)**
MoM <2.5 centile (%)	89 (2.5)	10 (5.0)*
Free triiodothyronine		
MoM (median, IQR)	0.991 (0.935-1.059)	0.979 (0.931-1.054)
pmol/L (median, IQR)	4.6 (4.4-5.0)	4.6 (4.3-4.9)
MoM <2.5 centile (%)	89 (2.5)	8 (4.0)

Comparison between the fetal loss and normal groups was by Chi square or Fisher exact test for categorical variables and Mann Whitney-U test for continuous variables. *p<0.05, **p<0.0001

Multiple logistic regression analysis demonstrated that in the prediction of fetal loss there were significant contributions from Afro-Caribbean racial origin (OR 4.102, 95% CI 3.003-5.603, p<0.001), use of ovulation drugs (OR 8.238, 95% CI 5.210-13.028, p<0.001), BMI (OR 1.028, 95% CI 1.000- 1.057, p=0.05), and log FT4 MoM (OR 0.011, 95% CI 0.001-0.104, p<0.001), but not TSH MoM (p=0.208). If in the regression FT4 MoM is not included then TSH MoM becomes significant. This is presumably the consequence of the good correlation between FT4 MoM and TSH MoM. The

associations between TSH and FT3, TSH and FT4 and FT3 and FT4 in both the fetal loss and unaffected groups are shown in Table 5.3.

Table 5.3. Correlations between thyroid stimulating hormone TSH), free thyroxine (FT4) and free triiodothyronine **(**FT3) in the normal and fetal loss groups.

	Normal		Fetal loss	
Correlations	**r**	**p**	**r**	**p**
TSH with FT3	-0.182	<0.0001	-0.609	<0.0001
TSH with FT4	-0.245	<0.0001	-0.697	<0.0001
FT3 with FT4	0.476	<0.0001	0.662	<0.001

Antithyroid antibodies

In the normal group 726 (16.8%) of the 4,318 pregnancies were positive for one or both antithyroid antibodies (Chapter 3). In this study of pregnancies complicated by fetal loss, the prevalence of antithyroid antibody positivity was not significantly different (Table 5.4).

Table 5.4. Prevalence of antithyroid antibody positivity in the normal and fetal loss groups.
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Pregnancy	**n**	**Antibody positive**			
		Anti-TPO	**Anti-Tg**	**Both**	**Either**
Normal	4318	441 (10.2%)	593 (13.7%)	308 (7.1%)	726 (16.8%)
Fetal loss	202	17 (8.4%)	25 (12.4%)	11 (5.5%)	31 (15.3%)

Anti-TPO = anti-thyroperoxidase antibody, anti-TG = anti-thyroglobulin antibody
Comparison between the normal and fetal loss groups was by Chi square or Fisher exact test.

5.4 DISCUSSION

This study has demonstrated that in pregnancies resulting in miscarriage or fetal death during the second and third trimesters, compared to those with normal outcome, the

median maternal serum concentration of TSH is increased and FT4 is decreased and the incidence of high TSH and low FT4 is increased. In contrast, there were no significant differences between the groups in the median concentration of FT3 or in the incidence of antithyroid antibody positivity.

In the fetal loss group, compared to the normal outcome group, more women were of Afro-Caribbean racial origin, the median maternal BMI was higher and more pregnancies were conceived after ovulation induction. These findings are compatible with the results of previous studies on the rates of second-trimester miscarriage and fetal death. Willinger *et al.*, examined the stillbirth hazard in 5,138,122 singleton pregnancies from the National Center of Health Statistics and reported that in Black, compared to White women, the risk of fetal death at 20-23 weeks was 2.75 times higher and the risk of death at 39-40 weeks was 1.57 times higher (Willinger *et al.,* 2009).

Obesity is associated with an increased risk of several adverse outcomes. A systematic review reported that the rate of pregnancy loss before 20 weeks of gestation increases with maternal BMI (Metwally *et al.,* 2008). Similarly, a population-based cohort study demonstrated that the rate of late fetal death increases with maternal BMI (Cnattingius *et al.,* 1998).

There is a scarcity of reports on the outcome of pregnancies conceived after the use of ovulation induction drugs without in-vitro fertilization. Whether pregnancies conceived through assisted reproductive technology (ART) are at an increased risk of loss is inconclusive, and data on maternal age-, ART type-, and gestational age-specific risk of loss are limited. Farr et al, examined the outcome of 148,494 ART pregnancies and reported that the overall risk of pregnancy loss was 29% and the risk in both groups increased with maternal age. The risk of pregnancy loss in singletons after confirmation of a fetal heartbeat was about 15% which is slightly higher than in naturally conceived pregnancies. The risk of fetal loss was 3.4% after 12 weeks of gestation and 1.2% after 20 weeks (Farr, *et al.,* 2007).

In our study, the incidence of high TSH and/or low FT4 in the fetal loss group was higher than in the normal outcome group. The contradictory results of previous reports concerning the association between subclinical hypothyroidism and fetal death may be a consequence of the small number of cases of fetal loss in the hypothyroid group and methodological differences between the studies. The two studies reporting an increase in fetal loss in women with high TSH did not provide data on FT4 which may have been normal or decreased (Sahu *et al.,* 2010; Hallengren *et al.,* 2009). In contrast, the studies reporting no significant difference in fetal loss between the high and low TSH groups included only women with normal FT4 (Casey *et al.,* 2005; Cleary-Goldman *et al.,* 2008).

The incidence of antithyroid antibody positivity for either anti-TPO or anti-Tg in the fetal loss group was not higher than in the normal outcome group. We have previously reported that in the antibody positive group, compared to the antibody negative group, the median TSH was higher and the median FT3 and FT4 were lower and this effect was observed for both anti-TPO and anti-Tg antibodies (Chapter 3).

Our findings do not support the hypothesis that antithyroid antibodies exert a direct toxic effect on the pregnancy leading to fetal loss however the mechanism of fetal loss in the first trimester may be different to fetal losses in the second and third trimester. It is likely that the previously reported association between antithyroid autoimmunity and fetal loss (Stagnaro-Green and Glinoer 2004), may be mediated by an underlying thyroid dysfunction. A study of euthyroid patients undergoing assisted reproduction technologies (ART) reported that the pregnancy and delivery rates were not different in the anti-TPO antibody positive and negative groups (Negro *et al.,* 2007a). However, the antibody positive women who failed to become pregnant or miscarried had higher TSH levels before ART than in those with a normal outcome. Another study examined whether anti-TPO antibody positive patients have an increased risk of miscarriage and if this can be reduced by levothyroxine treatment (Negro *et al.,* 2006). They reported that the miscarriage rate in anti-TPO antibody positive women with no treatment (13.8%) was significantly higher than in antibody negative women (3.5%) or in antibody

positive women treated with levothyroxine starting from the first-trimester (2.4%) (Negro *et al.,* 2006).

The strengths of our study are firstly, examination of a large number of pregnancies resulting in miscarriage or fetal death, secondly, assessment of confounding factors in the prediction of fetal loss, including maternal characteristics and method of conception, thirdly, use of normal ranges of thyroid function corrected for maternal characteristics, including age, racial origin and BMI (Chapter 3), and fourthly, assessment of thyroid function in the first-trimester of pregnancy providing the option for therapeutic interventions in future studies to determine if the incidence of fetal loss can be reduced.

A retrospective study of pregnancies in women with primary hypothyroidism treated with levothyroxine reported that the miscarriage rate in those that at the time of conception had overt or subclinical hypothyroidism was about 65%, whereas in the euthyroid group there were no miscarriages (Abalovich *et al.,* 2002). The extent to which the fetal loss rate during the second and third-trimesters can be reduced by the treatment of women diagnosed with hypothyroidism at 11-13 weeks remains to be determined. The limitation of this study is that it does not include early miscarriages most of which occur before 11 weeks.

5.5 CONCLUSIONS

There are multiple causes of miscarriage and fetal death during the second and third trimesters of pregnancy. This study has demonstrated that previously undiagnosed hypothyroidism diagnosed at 11-13 weeks of gestation may be a contributing factor to about 5% of subsequent fetal losses. The extent to which the diagnosis of subclinical hypothyroidism and appropriate therapy can prevent fetal loss and the cost-effectiveness of such strategy remain to be determined.

Chapter 6 Thyroid function in pregnancies that develop preeclampsia

ABSTRACT

Objective To determine if maternal thyroid function in the first-trimester is altered in pregnancies that subsequently develop preeclampsia (PE).

Methods Mean arterial pressure (MAP), uterine artery pulsatility index (PI) and maternal serum thyroid stimulating hormone (TSH), free thyroxine (FT4) and free triiodothyronine (FT3) at 11-13 weeks of gestation were measured in 102 singleton pregnancies that subsequently developed PE and the values were compared to the results of 4,318 normal pregnancies.

Results In both the PE group that required delivery before 34 weeks (early-PE) and the late-PE group, compared to the unaffected group, the median MAP multiple of the normal median (MoM) and uterine artery PI MoM were significantly increased. In late-PE but not in early-PE, compared to the unaffected group, the median TSH MoM was significantly increased and the median FT4 MoM was decreased. Logistic regression analysis demonstrated that TSH MoM provided a significant contribution in the prediction of late-PE.

Conclusions: Impaired thyroid function may predispose to the development of late-PE and measurement of maternal serum TSH can improve the prediction of late-PE provided by a combination of factors in the maternal history and the measurements of MAP and uterine artery PI.

This chapter is based on: Ashoor G, Maiz N, Rotas M, Kametas NA and Nicolaides KH (2010) Maternal thyroid function at 11 to 13 weeks of gestation and subsequent development of preeclampsia. *Prenat Diagn*, 30:1032-8.

6.1 INTRODUCTION

6.1.1 Background

Preeclampsia (PE), which affects about 2% of pregnancies, can be divided into early-PE requiring delivery before 34 weeks and late-PE with the former being associated with a high incidence of fetal growth restriction, whereas in late-PE fetal growth is usually normal (see Chapter 1.5). Effective first-trimester screening for both early-PE and late-PE is provided by a combination of maternal demographic characteristics and medical history, uterine artery PI and maternal mean arterial pressure (MAP) (Poon *et al.*, 2009). Several studies have reported than in patients presenting with the clinical features of PE, thyroid function is disturbed with increase in maternal serum TSH and decrease in the levels of thyroid hormones (see Chapter 1.5). There is also contradictory evidence as to whether PE causes hypothyroidism or subclinical hypothyroidism predispose to the development of PE, rather than the other way round (see Chapter 1.5).

6.1.2 Objective

The aims of this study are to investigate further if the prevalence of maternal thyroid hypofunction at 11-13 weeks of gestation is higher in pregnancies that subsequently develop PE and if it is whether assessment of thyroid function can improve the prediction of PE provided by a combination of factors in the maternal history and the measurements of MAP and uterine artery PI.

6.2 PATIENTS AND METHODS

The study design and overall study population are described in Chapter 2.

In this study we measured the maternal serum concentrations of free triiodothyronine (FT3), free thyroxine (FT4), TSH, anti-thyroperoxidase (TPO) and anti-thyroglobulin (Tg) at 11-13 weeks in 102 singleton pregnancies that subsequently developed PE.

None of the PE patients had a history of thyroid disease. The values were compared to the results of our normal group of 4,318 singleton pregnancies with no history of thyroid disease, which did not develop PE and resulted in live birth after 34 weeks of phenotypically normal neonates with birth weight above the 5th centile (Chapter 3). The normal group included 726 (16.8%) pregnancies in which the concentration of one or both antithyroid antibodies was 60 U/mL or more. Normal ranges for TSH, FT3 and FT4 were derived from the study of the 3,592 pregnancies with no antithyroid antibodies (Chapter 3).

Outcome measures

The definition of PE was that of the International Society for the Study of Hypertension in Pregnancy (Davey and MacGillivray, 1988). The diastolic blood pressure should be 90 mmHg or more on at least two occasions four hours apart developing after 20 weeks of gestation in previously normotensive women. In addition there should be proteinuria of 300 mg or more in 24 hours or two readings of at least ++ on dipstick analysis of midstream or catheter urine specimens if no 24-hour collection is available. In PE superimposed on chronic hypertension significant proteinuria (as defined above) should develop after 20 weeks of gestation in women with known chronic hypertension (history of hypertension before conception or the presence of hypertension at the booking visit before 20 weeks of gestation in the absence of trophoblastic disease).

Sample analysis

The maternal serum concentrations of FT3, FT4 and TSH were measured by immunoassay as previously described in Chapter 2.

Statistical analysis

The characteristics of the early-PE, late-PE and the unaffected group used for the construction of normal ranges were compared by Mann Whitney test for continuous variables and Fisher's exact test or Chi-square test for categorical variables. The

measured MAP and uterine artery L-PI were converted to multiples of the expected normal median (MoM) corrected for fetal CRL, maternal age, BMI or weight, smoking, parity, racial origin and method of conception as previously described (Poon *et al*., 2009). Similarly, the measured concentrations of FT3, FT4 and TSH were converted to MoMs corrected for gestational age, maternal age, racial origin and body mass index (Chapter 3).

Comparison of MAP MoM, uterine artery L-PI MoM, TSH MoM, FT3 MoM and FT4 MoM between early-PE, late-PE and the unaffected group was by Mann Whitney-U test, with post-hoc Bonferroni correction (critical statistical significance $p<0.025$). The risks for early-PE and late-PE based on combinations of maternal factors, MAP and uterine artery L-PI were determined as previously described and were then logarithmically transformed (Poon *et al.*, 2009). Logistic regression analysis was used to determine if the log transformed risk based on maternal factors, MAP and uterine artery L-PI and TSH MoM had a significant contribution in predicting early-PE and late-PE. The performance of screening was determined by receiver operating characteristic (ROC) curves.

In the early-PE, late-PE and unaffected groups the Chi-square test was used to compare the proportion of cases with anti-TPO and anti-Tg antibodies and those with serum TSH above the 97.5th centile and serum FT3 and FT4 below the 2.5th centile.

The statistical software packages SPSS 16.0 (SPSS Inc.,Chicago, IL) and Medcalc (Medcalc Software, Mariakerke, Belgium) were used for the data analyses.

6.3 RESULTS

The patient characteristics of the early-PE, late-PE and normal groups are compared in Table 6.1. In both the early-PE and late-PE groups, compared to the normal group, there was a higher prevalence of Afro-Caribbean women, chronic hypertensives and women with a personal or family history of PE. In women that developed late-PE the BMI was increased.

Table 6.1. Maternal demographic characteristics in the three outcome groups.

Variables	Normal (n=3,592)	Early PE (n=25)	Late PE (n=77)
Gestation at sampling in wks (median, IQR)	12.4 (12.3-12.9)	12.7 (12.1-13.1)	12.4 (12.1-12.7)
Gestation at delivery in wks (median, IQR)	40.0 (39.0-40.9)	32.4 (29.1-33.2)*	38.6 (37.3-39.9)*
Maternal age in yrs (median, IQR)	32.2 (28.0-36.0)	29.5 (23.7-34.9)	32.3 (27.4-37.2)
Body mass index in Kg/m^2, median (IQR)	24.7 (22.2-27.9)	25.1 (22.5-30.8)	27.8 (23.7-31.2)*
Racial origin			
White, n (%)	2,543 (70.8)	9 (36.0)	34 (44.2)
Black, n (%)	708 (19.7)	13 (52.0)*	34 (44.2)*
South Asian, n (%)	148 (4.1)	1 (4.0)	3 (3.9)
East Asian, n (%)	57 (1.6)	0	2 (2.6)
Mixed, n (%)	136 (3.8)	2 (8.0)	4 (5.2)
Parity			
Nulliparous, n (%)	1684 (46.9)	12 (48.0)	44 (57.1)
Parous – no previous preeclampsia, n (%)	1818 (50.6)	8 (32.0)	20 (26.0)*
Parous – previous preeclampsia, n (%)	90 (2.5)	5 (20.0)*	13 (16.9)*
Cigarette smoker, n (%)	322 (9.0)	1 (4.0)	5 (6.5)
Family history of preeclampsia, n (%)	128 (3.6)	5 (20.0)*	9 (11.7)*
Conception by ovulation drugs, n (%)	101 (2.8)	2 (8.0)	5 (6.5)
Chronic hypertension, n (%)	38 (1.1)	3 (12.0)*	5 (6.6%)*

Comparison between each hypertensive disorder group and unaffected was by Chi square or Fisher exact test for categorical variables and Mann Whitney-U test for continuous variables, both with post-hoc Bonferroni correction (critical statistical significance p<0.025): *p<0.025,
IQR: interquartile range

In both the early-PE and late-PE groups, compared to the normal group, the median MAP MoM and uterine artery L-PI MoM were significantly increased (Table 6.2). In late-PE but not in early-PE, compared to the unaffected group, the median TSH MoM was significantly increased and the median FT4 MoM was decreased. In late PE the proportion of cases with high TSH, low FT4 or low FT3 was higher than in the controls (Table 6.2, Figure 6.1).

Regression analysis demonstrated that in the PE group there were no significant associations between TSH MoM and uterine artery L-PI MoM (p=0.315), or between TSH MoM and MAP MoM (p=0.533).

Logistic regression analysis demonstrated that in the prediction of late-PE there were significant contributions from TSH MoM to the prediction from the combination of maternal factors, uterine artery L-PI and MAP. The areas under the ROC curves and detection rates for fixed false positive rates of 5% and 10% are given in Table 6.3.

Table 6.2. Mean arterial pressure, uterine artery lowest pulsatility index L-PI) and maternal serum thyroid stimulating hormone, free thyroxine and free triiodothyronine in the normal group and in those who subsequently developed early and late preeclampsia.

	Normal	Early preeclampsia	Late preeclampsia
Mean arterial pressure			
MoM (median, IQR)	0.98 (0.93-1.04)	1.06 (1.01-1.15)**	1.07 (1.00-1.13)**
mmHg (median, IQR)	84.67 (79.83-89.5)	91.3 (88.0-101.9)	94.4 (87.0-99.5)
Uterine artery L-PI			
MoM (median, IQR)	1.01 (0.82-1.22)	1.54 (1.17-1.64)**	1.20 (0.87-1.50)*
units (median, IQR)	1.41 (1.14-1.71)	2.10 (1.67-2.34)	1.68 (1.21-2.13)
Thyroid stimulating hormone			
MoM (median, IQR)	1.007 (0.608-1.511)	1.08 (0.556-1.781)	1.390 (0.708-2.122)*
m IU/L (median, IQR)	1.096 (0.670-1.665)	1.094 (0.500-1.583)	1.357 (0.730-2.346)
>97.5 centile (%)	89 (2.5)	2 (8.0)	10 (13.0)*
Free thyroxine			
MoM (median, IQR)	0.992 (0.908-1.086)	0.966 (0.868-1.043)	0.951 (0.843-1.052)*
Pmol/L (median, IQR)	14.9 (13.6-16.3)	14.1 (13.1-15.5)	14.0 (12.4-15.6)
% <2.5 centile (%)	89 (2.5)	1 (4.0)	6 (7.8)*
Free triiodothyronine			
MoM (median, IQR)	0.991 (0.935-1.059)	0.954 (0.881-1.054)	1.010 (0.934-1.064)
Pmol/L (median, IQR)	4.6 (4.4-5.0)	4.5 (4.1-5.0)	4.7 (4.4-5.0)
% <2.5 centile (%)	89 (2.5)	2 (8.0)	6 (7.8)*

Comparison between each hypertensive disorder group and unaffected was by Chi square or Fisher exact test for categorical variables and Mann Whitney-U test for continuous variables, both with post-hoc Bonferroni correction (critical statistical significance $p<0.025$): *$p<0.025$, **<0.001
IQR: interquartile range

Antithyroid antibodies

In normal pregnancy 726 (16.8%) of the 4,318 pregnancies were positive for one or both antithyroid antibodies, 308 (7.1%) were positive for both, 285 (6.6%) were positive for anti-Tg only and 133 (3.1%) were positive for anti-TPO antibody (Chapter 3).

In this study of pregnancies complicated by PE the prevalence of antithyroid antibody positivity was not significantly increased; in late-PE 11 (14.3%) of the 77 pregnancies

had positive antibodies, 6 (7.8%) were positive for both, 4 (5.2%) were positive for anti-Tg and 1 (1.3%) was positive for anti-TPO; in early-PE 3 (12.0%) of the 25 pregnancies (p=0.687) were positive for both antibodies, 2 (8%) were anti-Tg positive and 1 (4%) was anti-TPO positive.

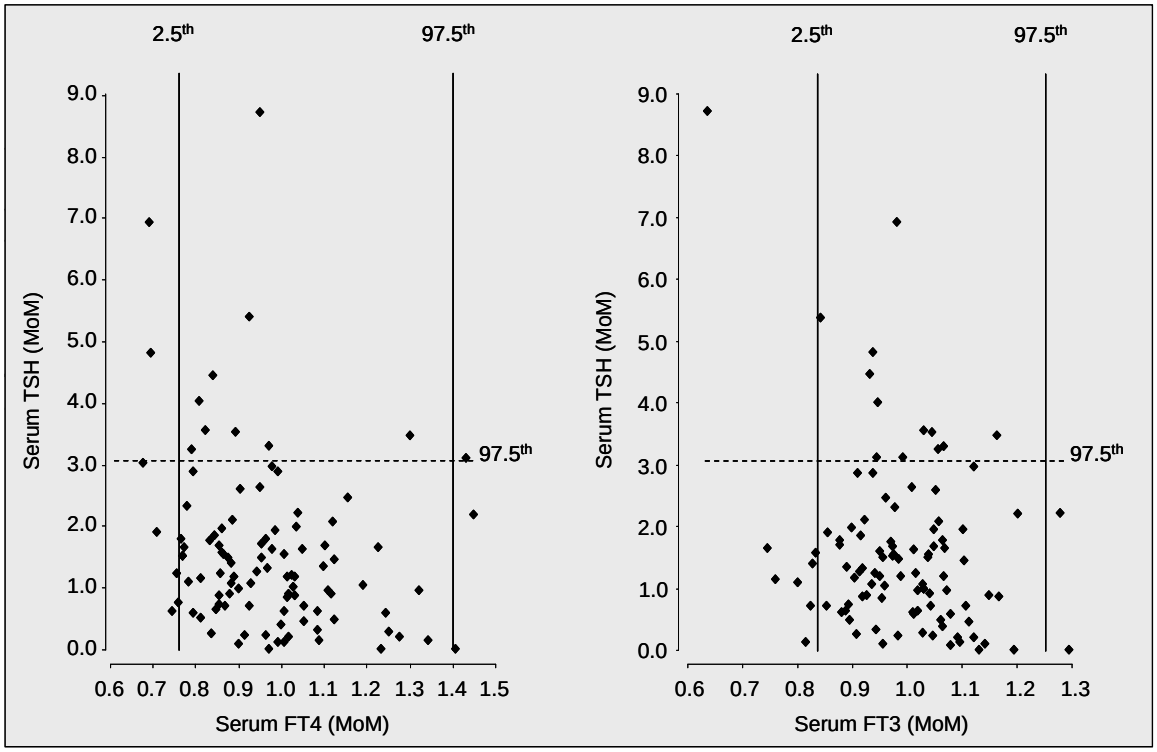

Figure 6.1. Relationship between maternal serum thyroid stimulating hormone (TSH) and free thyroxine (FT4) and free triidothyronine (FT3) in multiples of the expected normal median (MoM) at 11-13 weeks of gestation in pregnancies that subsequently developed preeclampsia. The vertical lines represent the 2.5th and 97.5th centiles of the normal ranges for FT4 and FT3 and the interrupted horizontal lines the 97.5th centile for TSH.

6.4 DISCUSSION

The findings of this study demonstrate an association between impaired maternal thyroid function at 11-13 weeks and subsequent development of late PE. High serum TSH was observed in 5 times as many with late-PE compared with those who did not develop PE. This association of hypothyroidism and PE is independent of autoimmune

mechanisms because the prevalence of antithyroid antibodies was not higher in the PE than in the non-PE group. A study of 5505 patients examining early pregnancy serum samples for thyroid function reported that in the group with subclinical hypothyroidism the incidence of subsequent development of PE was higher than in the euthyroid group (3.8 vs 1.9%) but this difference did not reach statistical significance (Mannisto *et al*., 2010). Another first trimester screening study involving 10990 patients reported that the subclinical hypothyroidism group was not associated with the development of PE but the reported prevalence of PE in this study was only 1% (Cleary-Goldman *et al.,* 2008). These studies did not report separately their findings of early and late PE (Mannisto *et al*., 2010 and Cleary-Goldman *et al.,* 2008).

Table 6.3. Performance of screening for late preeclampsia by maternal factors only, TSH MoM, a combination of maternal factors with TSH MoM, a combination of maternal factors, lowest uterine artery pulsatility index (L-PI) and mean arterial pressure (MAP) and a combination of maternal factors, uterine artery L-PI, MAP and TSH MoM.

Screening test	**Area under receiver operating characteristic curve (95% CI)**	
	Late preeclampsia	
Maternal risk factor	0.785 (0.770-0.799)	
TSH MoM	0.603 (0.586-0.621)	
Maternal risk factor plus		
TSH	0.793 (0.778-0.807)	
MAP, uterine artery L-PI	0.856 (0.843-0.868)	
All markers	0.860 (0.848-0.872)	
	Detection rate (%) for fixed false positive rate (95% CI)	
	5%	**10%**
Maternal risk factor	27.5 (17.5-39.6)	37.7 (26.3-50.2)
TSH MoM	21.7 (12.7-33.3)	27.5 (17.5-39.6)
Maternal risk factor plus		
TSH	36.2 (25.0-48.7)	47.8 (35.6-60.2)
MAP, uterine artery L-PI	39.1 (27.6-51.6)	53.6 (41.2-65.7)
All markers	43.5 (31.6-56.0)	59.4 (46.9-71.1)

Our results raise the possibility that the findings of the population based study in which serum TSH was measured in women 20 years after their pregnancies and found to be higher in those who had developed PE (Levine *et al*., 2009b), may merely reflect a preexisting thyroid dysfunction that preceded their pregnancies. Another population based cohort with follow up for 20 years reported that thyroid dysfunction in early pregnancy was not associated with the development of PE but those with subclinical hypothyroidism were at increased risk of developing overt hypothyroidism in the long term (Mannisto *et al*., 2010).

The study has confirmed the association between the development of PE and factors in the maternal history and the measurements of MAP and uterine artery PI (Poon *et al.*, 2009). Increased risk for both early-PE and late-PE was observed in women of Black racial origin and those with a family or personal history of PE and chronic hypertension. Late-PE was also associated with increased BMI. In both early-PE and late-PE, MAP and uterine artery PI were increased but the increase in PI was more pronounced in those with early-PE. Impaired thyroid function was more pronounced in pregnancies that developed late-PE than early-PE.

The strengths of this study are firstly, examination of a large number of appropriately documented cases of PE and normal controls, secondly, assessment of confounding factors in the prediction of PE, including maternal history, MAP and uterine artery PI, thirdly, use of normal ranges of thyroid function corrected for maternal characteristics, including age, racial origin and body mass index (Chapter 3), and fourthly, assessment of thyroid function in the first-trimester of pregnancy providing the option for therapeutic interventions in future studies to determine if the incidence of PE can be reduced. The weakness of the study was its cross-sectional nature, which did not allow longitudinal assessment of thyroid function from early pregnancy to the development of PE.

There is extensive evidence that the underlying mechanism for early-PE is impaired trophoblastic invasion of the maternal spiral arteries, reduced placental perfusion and fetal growth restriction (Yu *et al.,* 2008; Plasencia *et al.,* 2007; Poon *et al.,* 2009). In

contrast, in late-PE placental perfusion and fetal growth are often normal and the main pathophysiological processes resemble those of the metabolic syndrome with an increase in adipose tissue and impaired glucose and lipid metabolism (Witlin *et al*., 2000; Poon *et al.,* 2009; Moldenhauer *et al*., 2003; Egbor *et al.,* 2006; Vatten *et al.,* 2004; D'Anna *et al*., 2006). The association between hypothyroidism and late-PE may be mediated by the well described role of thyroid hormones in glucose homeostasis and in the synthesis, metabolism and mobilization of lipids (Chidakel *et al*., 2005; Duntas, 2002; Pearce, 2004). Hypothyroidism may also play a direct role in causing pregnancy hypertension because thyroid hormones act directly on peripheral arterioles to cause dilation (Dernellis and Panaretou, 2002). Studies in non-pregnant individuals reported that hypothyroidism is associated with an increase in peripheral resistance due to increased arterial wall thickness (Giannattasio *et al*., 1997) and endothelial dysfunction (Virdis *et al.,* 2009). This can be reversed by treatment with thyroid hormones (Dernellis and Panaretou, 2002; Giannattasio *et al*., 1997).

6.5 CONCLUSIONS

Measurement of maternal serum TSH can improve the prediction of late-PE provided by a combination of factors in the maternal history and the measurements of MAP and uterine artery PI. The ability to predict in very early pregnancy those women at high-risk for PE might decrease maternal and fetal morbidity through closer surveillance by physicians experienced or specialized in high-risk obstetrics, as well as delivery at tertiary care centres (Levine and Lindheimer, 2009a). Effective identification of the high-risk group can also be useful for future studies investigating the potential role of pharmacological interventions starting from the first-trimester to reduce the prevalence of the disease.

Chapter 7 Thyroid function in pregnancies delivering small for gestational age neonates

ABSTRACT

Background: *Studies have shown that altered thyroid function in early pregnancy may affect normal placental development and hence fetal growth. Our hypothesis is that maternal thyroid function in the first trimester is altered in pregnancies that subsequently deliver small for gestational age neonates (SGA).*

Methods: *Maternal serum thyroid stimulating hormone (TSH), free thyroxine (FT4) and free triiodothyronine (FT3) were measured at 11^{+0}-13^{+6} weeks' gestation in 212 singleton pregnancies with no history of thyroid disease that subsequently delivered SGA neonates and the values were compared to the results of 3,598 normal pregnancies delivering neonates with birth weight above the 5^{th} percentile for gestation.*

Results: *There were no significant differences between the normal and SGA group in median multiple of the median (MoM) TSH (1.07 vs 1.061 MoM), FT4 (0.992 vs 1.010 MoM) and FT3 (0.991 vs 0.990 MoM).*

Conclusion: *In women with no history of thyroid disease delivering SGA neonates thyroid function during the first trimester of pregnancy is not significantly different from women delivering non-SGA neonates.*

This chapter is based on: Karagiannis G, Ashoor G, Maiz N, Jawdat F and Nicolaides KH (2011) Maternal thyroid function at eleven to thirteen weeks of gestation and subsequent delivery of small for gestational age neonates. *Thyroid,* 21:1127-31.

7.1 INTRODUCTION

7.1.1 Background

Small-for-gestational age (SGA) neonates, with birthweight below the 5th percentile, is heterogeneous and includes constitutionally small neonates and growth restricted ones due to impaired placentation, genetic disease or environmental damage. Impaired trophoblastic invasion and placentation is thought to be the underlying mechanism for many cases of preeclampsia (PE) and of impaired fetal growth in the absence of PE (see Chapter 1.5). The mechanism underlying trophoblast proliferation and invasion is largely unknown but there is some evidence implicating thyroid hormones in this process (Maruo *et al.,* 1991; Barber *et al.,* 2005; Oki *et al.,* 2004). There is also contradictory evidence that clinical and subclinical hypothyroidism is associated with increased risk for both PE and the birth of SGA neonates in the absence of PE (see Chapter 1.5).

7.1.2 Objective

The aim of this study is to investigate further if the prevalence of maternal thyroid hypofunction at 11-13 weeks' of gestation is higher in pregnancies that subsequently delivered SGA neonates in the absence of PE.

7.2 PATIENTS AND METHODS

The study design and overall study population are described in Chapter 2.

In this study we measured the maternal serum concentrations of TSH, FT3 and FT4 at 11-13 weeks in 212 singleton pregnancies with no history of thyroid disease, which did not develop PE and resulted in live birth of phenotypically normal neonates with birth weight below the 5th percentile for gestational age (SGA group, Figure 7.1) (Poon *et al.,* 2011b).

At presentation none of the women had overt hypothyroidism or hyperthyroidism. The values were compared to those in 3,592 singleton pregnancies with no history of thyroid disease, which did not develop PE and resulted in live birth after 34 weeks of phenotypically normal neonates with birth weight above the 5th percentile (Chapter 3).

Sample analysis

The maternal serum concentrations of FT3, FT4 and TSH were measured by immunoassay as previously described in Chaper 2.

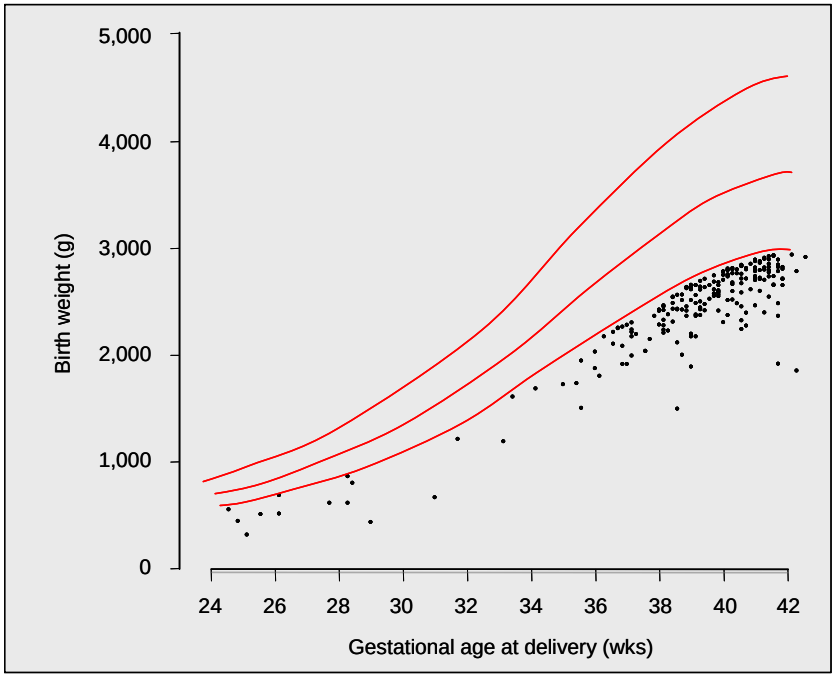

Figure 7.1. Distribution of birth weight of the small for gestational age fetuses (black dots) plotted on the reference range of birth weight for gestational age (median, 5th and 95th percentiles (red lines)

Statistical analysis

The characteristics of the SGA group with the unaffected group were compared by

Mann Whitney test for continuous variables and Fisher's exact test or Chi-square test for categorical variables. The measured concentrations of FT3, FT4 and TSH were converted to multiples of the expected normal median (MoM) corrected for gestational age and maternal age, racial origin and BMI (Chapter 3).

The SGA and unaffected groups were compared for median TSH MoM, FT3 MoM and FT4 MoM using the Mann Whitney test and for the proportion of cases with serum TSH above the 97.5th percentile and serum FT3 and FT4 below the 2,5th percentile by the Chi-square test. Regression analysis was also used to determine the significance of the interrelations between TSH MoM, FT3 MoM and FT4 MoM.

The statistical software packages SPSS 15.0 (SPSS Inc.,Chicago, IL) was used for the data analyses.

7.3 RESULTS

The patient characteristics of the SGA and unaffected groups are compared in Table 7.1. In the SGA group the maternal age and BMI were lower and the prevalence of African and South Asian women, cigarette smokers and those with chronic hypertension was higher.

In the SGA group, compared to the unaffected group, the median TSH MoM, FT3 MoM and FT4 MoM were not significantly different (Table 7.2) and there was no significant association between the gestational age at delivery and TSH MoM (p=0.662), FT3 MoM (p=0.538) and FT4 MoM (p=0.543).

The significance of the associations between TSH, FT3 and FT4 in the unaffected and SGA groups is shown in Table 7.3.

Table 7.1. Maternal demographic characteristics in the small and not-small for gestational age groups.

Variables	Not small for gestation (n=3,592)	Small for gestation (n=212)
Gestation: sampling in wks (median, IQR)	12.4 (12.3-12.9)	12.4 (12.1-12.9)
Gestation: delivery in wks (median, IQR)	40.0 (39.0-40.9)	39.6 (38.2-40.9)*
Maternal age in yrs (median, IQR)	32.2 (28.0-36.0)	31.0 (25.1-35.7)*
Body mass index in Kg/m^2, median (IQR)	24.7 (22.2-27.9)	23.1 (21.1-26.4)**
Ancestral origin		
Caucasian, n (%)	2,543 (70.8)	124 (58.5)
African, n (%)	708 (19.7)	58 (27.4)*
South Asian, n (%)	148 (4.1)	26 (7.5)**
East Asian, n (%)	57 (1.6)	3 (1.4)
Mixed, n (%)	136 (3.8)	11 (5.2)
Parity		
Nulliparous, n (%)	1684 (46.9)	130 (61.3)
Parous, n (%)	1908 (53.1)	82 (38.7)**
Cigarette smoker, n (%)	322 (9.0)	45 (21.2)**
Conception by ovulation drugs	101 (2.8)	5 (2.4)
Chronic hypertension	38 (1.1)	9 (4.2)*

Comparison between the two groups was by Chi square or Fisher exact test for categorical variables and Mann Whitney-U test for continuous variables. *p<0.05, ** p<0.0001

Table 7.2. Maternal serum TSH, FT3, FT4 values in the small and not-small for gestational age groups.

	Not small for gestation (n=3592)	Small for gestation (n=212)
Thyroid stimulating hormone		
MoM (median, IQR)	1.007 (0.608-1.511)	1.061 (0.651-1.562)
mIU/L (median, IQR)	1.096 (0.670-1.665)	1.107 (0.690-1.687)
MoM >97.5 centile (%)	89 (2.5)	10 (4.7)
Free thyroxine		
MoM (median, IQR)	0.992 (0.908-1.086)	1.010 (0.919-1.090)
pmol/L (median, IQR)	14.9 (13.6-16.3)	15.2 (13.7-16.4)
MoM <2.5 centile (%)	89 (2.5)	6 (2.8)
Free triiodothyronine		
MoM (median, IQR)	0.991 (0.935-1.059)	0.990 (0.940-1.063)
pmol/L (median, IQR)	4.6 (4.4-5.0)	4.7 (4.4-5.0)
MoM <2.5 centile (%)	89 (2.5)	4 (1.9)

Comparison between the the two groups was by Chi square or Fisher exact test for categorical variables and Mann Whitney-U test for continuous variables. *p<0.05,

Table 7.3. Associations between TSH, FT3 and FT4 in the unaffected and small for gestational age groups.

	Unaffected		Small for gestational age	
Correlations	**r**	**p**	**r**	**p**
TSH with FT3	-0.182	<0.0001	-0.259	<0.0001
TSH with FT4	-0.245	<0.0001	-0.133	0.052
FT3 with FT4	0.476	<0.0001	0.304	<0.0001

7.4 DISCUSSION

The findings of this study indicate that in pregnancies delivering SGA neonates, maternal thyroid function at 11-13 weeks gestation is not significantly different from those delivering appropriately grown neonates and there is no evidence that in the SGA group the incidence of impaired thyroid function is increased.

A previous screening study in which maternal thyroid function was assessed at 15-18 weeks' gestation reported that there was no significant difference in mean birth weight between euthyroid pregnancies and those with subclinical hypothyroidism (Allan *et al.,* 2000). In contrast, a case control study reported that the mean birth weight in pregnancies with subclinical hypothyroidism was significantly lower than in euthyroid controls (Blazer *et al.,* 2003).

Another screening study before 20 weeks reported that the incidence of neonates with birth weight below 2.5 Kg was not significantly different between euthyroid pregnancies and those with subclinical hypothyroidism (Casey *et al.,* 2007). However, use of mean birth weight or a cut-off in birth weight without appropriate adjustments for gestational age are not appropriate for the investigation of thyroid function on fetal growth.

The strengths of our study are firstly, examination of a large number of appropriately documented cases of SGA and normal controls, secondly, comparison of the SGA and non-SGA groups after adjustment of the results of thyroid function test for those factors found to affect measurements in normal pregnancies, including gestational

age, maternal age, racial origin and BMI (Chapter 3), and thirdly, assessment of thyroid function in the first-trimester of pregnancy providing the option for therapeutic interventions in future studies to determine if the incidence of SGA can be reduced. The weakness of the study was its cross-sectional nature, which did not allow longitudinal assessment of thyroid function from early pregnancy to the development of SGA.

The findings that firstly, SGA is not associated with maternal thyroid hypofunction and secondly, there is no correlation between gestational age at delivery and TSH, FT3 or FT4 suggest that the results of *in vitro* studies concerning the role of thyroid hormones on trophoblast proliferation and invasion (Barber *et al.,* 2005; Oki *et al.,* 2004) may not be clinically relevant. Histological studies reported that impaired placentation is observed in all cases of PE with or without SGA and in about half of pregnancies with SGA in the absence of PE (Brosens *et al.,* 1977). However, Doppler studies of the uterine arteries documented that the prevalence of high impedance to flow in pregnancies with PE depends on the gestational age at the onset of the disease. The uterine artery pulsatility index (PI) was above the 95th percentile in 82% of those that developed PE requiring delivery before 34 weeks (early-PE) and in 40% of those delivering at or after 34 weeks (late-PE) (Yu *et al.,* 2008). Similarly, in SGA without PE high PI was observed in 44% of those that delivered before 34 weeks and in 10% of those delivering at or after 34 weeks.

Although the basis of the possible association between hypothyroidism, PE and SGA was the suggested role of thyroid hormones in promoting placentation and trophoblastic invasion, we previously found that thyroid hypofunction is observed in pregnancies developing late- rather than early-PE (Chapter 6).

We therefore suggested that the association is unlikely to be mediated by impaired trophoblastic invasion but rather by a metabolic derangement with increased insulin resistance, which is thought to underlie late-PE (Egbor *et al.,* 2006; Moldenhauer *et al.,* 2003; Kaaja *et al.,* 1999; Bosio *et al.,* 1999; D'Anna *et al.,* 2006).

7.5 CONCLUSIONS

In pregnancies delivering SGA neonates maternal thyroid function at 11-13 weeks' gestation is not impaired. Consequently, irrespective of the possible effect of thyroid hormones on placentation, in women with no history of thyroid disease, thyroid function does not have a significant contribution to the prevalence of SGA neonates.

Chapter 8 Thyroid function in pregnancies resulting in spontaneous preterm delivery

ABSTRACT

Objective: To estimate the possible association between spontaneous early preterm delivery and maternal thyroid dysfunction in early pregnancy.

Methods: Maternal serum concentrations of thyroid-stimulating hormone (TSH), free triiodothyronine (FT3), free thyroxine (FT4), anti-thyroperoxidase (anti-TPO) and anti-thyroglobulin (anti-Tg) antibodies at 11-13 weeks' gestation were compared in 102 singleton pregnancies resulting in spontaneous delivery before 34 weeks and 4,318 normal pregnancies delivering after this gestation.

Results: In the preterm delivery group, compared to the normal outcome group, there was no significant difference in anti-thyroid antibody positivity (16.7 vs. 16.8%). In the anti-thyroid antibody negative pregnancies in the preterm delivery group, compared to the normal outcome group, the median FT3 multiple of the normal median (MoM) and FT4 MoM were reduced (0.97 and 0.94 vs. 0.99 MoM, $p<0.05$ and $p<0.001$, respectively) but the median TSH MoM was not significantly different (0.99 vs. 1.01 MoM, $p=0.331$).

Conclusions: In pregnancies resulting in spontaneous early preterm delivery there is no evidence of increased prevalence of anti-thyroid antibody positivity or maternal thyroid dysfunction at 11-13 weeks.

This chapter is based on: Ashoor G, Maiz N, Rotas M, Jawdat F, Nicolaides KH 2011 Maternal thyroid function at 11-13 weeks of gestation and spontaneous preterm delivery. Obstet Gynecol 117:293-8.

8.1 INTRODUCTION

8.1.1 Background

Preterm delivery is the leading cause of perinatal death and there is contradictory evidence that both subclinical hypothyroidism and autoimmune thyroid disease in euthyroid women are associated with preterm delivery (see Chapter 1.5).

8.1.2 Objective

The aim of this study is to estimate the possible association between maternal thyroid dysfunction and preterm delivery by comparing anti-thyroid antibody positivity and serum TSH, FT3 and FT4 levels at 11-13 weeks' gestation, after appropriate adjustments for maternal characteristics, in pregnancies which subsequently resulted in spontaneous delivery before 34 weeks with normal pregnancies delivering after this gestation.

8.2 PATIENTS AND METHODS

The study design and overall study population are described in Chapter 2.

In this study we retrospectively examined maternal thyroid function and anti-thyroid antibodies at 11-13 weeks in 102 singleton pregnancies with no history of thyroid disease, resulting in spontaneous preterm delivery before 34 weeks' gestation of phenotypically normal neonates (preterm delivery group).

The values were compared to those of 4,318 normal singleton pregnancies with no history of thyroid disease, resulting in live birth after 34 weeks of phenotypically normal neonates (normal outcome group) (Chapter 3). Pregnancies complicated by preeclampsia were excluded from both the preterm delivery and normal outcome groups.

Sample analysis

The maternal serum concentrations of FT3, FT4 and TSH were measured by immunoassay as previously described in Chapter 2.

Statistical analysis

Comparison between the preterm delivery and normal outcome groups was by χ^2-test or Fisher's exact test for categorical variables and by Mann Whitney test for continuous variables. In the anti-thyroid antibody negative pregnancies (anti-TPO and anti-Tg level of less than 60 U/mL) the measured concentrations of FT3, FT4 and TSH were logarithmically transformed. However, $\log_{10}$ TSH remained negatively skewed therefore square root ($\sqrt{}$) transformation was applied. Histograms and probability plots showed that the distributions of $\sqrt{}$ TSH, logFT3 and logFT4 were normal. The values were then expressed as multiples of the expected normal median (MoM) corrected for gestational age and maternal age, racial origin and body mass index.

The preterm delivery and normal outcome groups were compared for median TSH MoM, FT3 MoM and FT4 MoM and prevalence of anti-thyroid antibodies. Pearson correlation was used to determine the significance of the interrelations of $\sqrt{}$ TSH MoM with $\log_{10}$ FT3 MoM and $\log_{10}$ FT4 MoM. Non parametric Spearman's correlation coefficient was used to estimate the association between $\sqrt{}$ TSH MoM, $\log_{10}$ FT3 MoM and $\log_{10}$ FT4 MoM with gestational age at delivery in the preterm delivery group.

The statistical software package PASW statistics 18.0 (SPSS Inc.,Chicago, IL) was used for the data analyses.

8.3 RESULTS

The patient characteristics of the preterm delivery and normal outcome groups are compared in Table 8.1. In the preterm delivery group there was a higher prevalence of

women of African racial origin and those conceiving after ovulation induction.

In the preterm delivery group, compared to the normal outcome group, there was no significant difference in anti-thyroid antibody positivity (Table 8.2). In the anti-thyroid antibody negative pregnancies in the preterm delivery group, compared to the normal outcome pregnancies, the median FT4 MoM and FT3 MoM were reduced ($p<0.05$ and $p<0.001$, respectively) but the median TSH MoM was not significantly different ($p=0.331$; Figure 8.1, Table 8.3).

Table 8.1. Characteristics of the spontaneous preterm delivery and normal outcome groups.

Variables	**Normal outcome n=4,318**	**Preterm delivery n=102**
Gestation at sampling in wks (median, IQR)	12.4 (12.3-12.9)	12.6 (12.3-13.0)
Gestation at delivery in wks (median, IQR)	40.2 (39.2-41.0)	31.4 (29.4-33.0)**
Maternal age in yrs (median, IQR)	32.4 (28.2-36.1)	32.2 (26.8-36.2)
Body mass index in Kg/m^2, median (IQR)	24.6 (22.3-27.9)	25.0 (21.8-28.1)
Racial origin		
Caucasian, n (%)	3,125 (72.4)	60 (58.8)
African, n (%)	759 (17.6)	31 (30.4)*
Indian or Pakistani, n (%)	211 (4.9)	6 (5.9)
Chinese or Japanese, n (%)	70 (1.6)	0
Mixed, n (%)	153 (3.5)	5 (4.9)
Parity		
Nulliparous, n (%)	2,046 (47.4)	46 (45.1)
Parous, n (%)	2,272 (52.6)	56 (54.9)
Cigarette smoker, n (%)	361 (8.4)	13 (12.7)
Conception by ovulation drugs	128 (3.0)	9 (8.8)*

Comparison between the spontaneous preterm delivery and the normal outcome groups was by Chi square or Fisher exact test for categorical variables and *t*- test for continuous variables. *$p<0.05$, **$p<0.0001$

Table 8.2. Prevalence of antithyroid antibody positivity in the two pregnancy outcome groups.

Outcome group	**n**	**Antibody positive**			
		Anti-TPO	**Anti-Tg**	**Both**	**Either**
Normal outcome	4,318	441 (10.2%)	593 (13.7%)	308 (7.1%)	726 (16.8%)
Preterm delivery	102	12 (11.8%)	10 (9.8%)	5 (4.9%)	17 (16.7%)

In the preterm delivery group serum TSH was above the 95th percentile of the normal range in 1 (1.2%) case and serum FT3 and FT4 were below the 5th percentile in 7

(8.2%) and 8 (9.4%) cases, respectively and they were not significantly different from the normal outcome group (p=0.128, p=0.202 and p=0.077, respectively).

Table 8.3. Maternal serum thyroid-stimulating hormone free triiodothyronine and free thyroxine in the spontaneous preterm delivery and normal outcome groups.

	Normal outcome (n=3,592)	**Preterm delivery (n=85)**
Thyroid-stimulating hormone		
MoM (median, IQR)	1.01 (0.61-1.51)	0.99 (0.57-1.42)
mIU/L (median, IQR)	1.10 (0.67-1.67)	0.99 (0.56-1.51)
Free thyroxine		
MoM (median, IQR)	0.99 (0.91-1.09)	0.94 (0.84-1.03)**
pmol/L (median, IQR)	14.90 (13.60-16.30)	14.10 (12.45-15.35)**
Free triiodothyronine		
MoM (median, IQR)	0.99 (0.93-1.06)	0.97 (0.91-1.05)*
pmol/L (median, IQR)	4.63 (4.40-5.00)	4.50 (4.20-4.90)*

Comparison between the spontaneous preterm delivery and the normal outcome groups was by Mann Whitney test. **p<0.05, **p<0.001

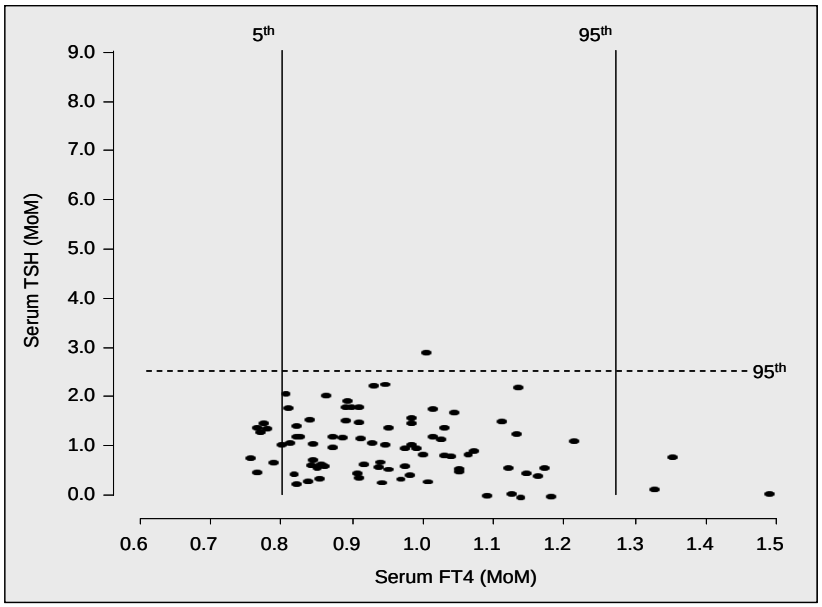

Figure 8.1. Relationship between maternal serum thyroid stimulating hormone (TSH) and free thyroxine (FT4) in multiples of the expected normal median (MoM) at 11-13 weeks of gestation in pregnancies that subsequently resulted in spontaneous delivery before 34 weeks. The vertical lines represent the 5th and 95th centiles of the normal ranges for FT4 and the interrupted horizontal lines the 95th centile for TSH.

There were significant associations between √ TSH MoM and log_{10} FT3 MoM in the normal outcome group (r=-0.182, p<0.0001) but not in the preterm delivery group (r=-0.205, p=0.060). There were significant associations between √ TSH MoM and log_{10} FT4 MoM log_{10} in both the preterm delivery (r=-0.329, p=0.002) and normal outcome (r=-0.245, p<0.0001) groups. In the preterm delivery group there was no significant association between gestation at delivery and √ TSH MoM (r=-0.053, p= 0.629), or log_{10} FT4 MoM (r=0.061, p=0.579), but there was an association with log_{10} FT3 MoM (r=-0.279, p=0.010).

8.4 DISCUSSION

The findings of this study demonstrate that in pregnancies resulting in spontaneous early preterm delivery there is no evidence of increased prevalence of anti-thyroid antibody positivity or maternal thyroid dysfunction at 11-13 weeks.

Preterm birth is the leading cause of perinatal death and handicap in children and the vast majority of mortality and morbidity relates to early delivery before 34 weeks (Saigal *et al.* 2008; Centre for Maternal and Child Enquiries, 2010). Delivery before 34 weeks occurs in about 2% of singleton pregnancies and in two-thirds of the cases this is due to spontaneous onset of labor or preterm prelabor rupture of membranes and in the other one-third it is iatrogenic, mainly due to preeclampsia (Celik *et al.* 2008). Consequently, in investigating the possible association maternal thyroid dysfunction and preterm delivery we firstly, excluded cases of iatrogenic preterm delivery and secondly, selected those delivering before 34 than 37 weeks because they have a worse pregnancy outcome.

A previous study of 28 pregnancies delivering before 32 weeks reported that at 15 weeks' gestation the maternal serum TSH was above the 97.5th centile in a significantly higher proportion of cases than in 124 pregnancies delivering at term (14 vs 6%) (Stagnaro-Green *et al.* 2005). However, in this study 64% of the cases with early preterm delivery had hypertensive disorders of pregnancy and it is therefore

uncertain whether it is spontaneous preterm delivery or hypertensive disease that is associated with thyroid dysfunction.

In women of African racial origin the rate of spontaneous early preterm delivery was higher than in Caucasians. This is compatible with the results of previous studies. National statistics in the USA demonstrate that the risk of preterm delivery in women of African racial origin is 1.6 times higher than in Caucasians (Mathews and MacDorman, 2008). Similarly, a population-based study of 585,291 singleton pregnancies from North London, UK, reported that after correcting for other confounders the risk of spontaneous delivery before 37 weeks was higher by 1.6 times for women of African racial origin compared to Caucasians (Balchin *et al.* 2007). As for the association between preterm delivery and the use of ovulation induction drugs some studies suggest a method-related cause and others that infertility rather than its treatment is the cause because infertile women being older are more likely to suffer from chronic medical conditions (Wang *et al.* 2005; Filicori *et al.* 2005; Blickstein, 2006).

The prevalence of anti-thyroid antibody positivity in women with spontaneous early preterm delivery was not higher than in those with normal pregnancy outcome. This finding is in agreement with the results of a screening study for anti-thyroid antibodies in early pregnancy which found that the rate of preterm delivery was not significantly different between the antibody positive and negative pregnancies (IIjima *et al.*1997). In contrast, some studies reported that the rate of preterm delivery in euthyroid women with anti-Tg and / or anti-TPO antibodies was 2-3 times higher than in women with no anti-thyroid antibodies (Glinoer *et al.*1994; Ghafoor *et al.* 2006). Additionally, a study of euthyroid women positive for anti-TPO antibodies reported that the administration of levothyroxine during pregnancy, compared to no treatment, was associated with significant reduction in the rate of preterm delivery (7% vs 22%) (Negro *et al.* 2006).

Serum TSH was not higher in the preterm delivery group than in the normal outcome group. This finding is compatible with the results of a screening study in 9,404 pregnancies at 15-18 weeks which reported that there was no significant difference in mean gestation at delivery between those with TSH at or above 6 mU/L and those with

TSH below 6 mU/L (Allan *et al.* 2000). The results are also compatible with the findings of the first-trimester screening study of Cleary-Goldman *et al.* (2008), where the rate of delivery before 37 weeks in women with subclinical hypothyroidism was not significantly different than in euthyroid pregnancies. In contrast, the screening study of Casey *et al.* (2005), in which thyroid function was assessed before 20 weeks' gestation, reported that in women with subclinical hypothyroidism there was a doubling in the rate of delivery before 34 weeks.

In the spontaneous early preterm delivery group the median serum FT3 and FT4 concentration was reduced but the incidence of FT3 and FT4 below the 5th centile was not significantly different from the incidence in the normal outcome group. In both the studies of Cleary-Goldman *et al.* (2008) and Casey *et al.* (2007), isolated maternal hypothyroxinemia, defined by TSH between the 2.5th and 97.5th percentiles and FT4 below the 2.5th percentile, was not associated with increased risk of preterm delivery.

The strengths of our study are firstly, distinction between spontaneous and iatrogenic preterm delivery, secondly, adjustment of measured concentrations of serum TSH, FT3 and FT4 for the maternal factors known to affect these measurements and thirdly, examination of a large number of spontaneous early preterm deliveries.

8.5 CONCLUSIONS

We found that there is no significant difference between the preterm delivery and normal outcome groups in the prevalence of anti-thyroid antibody positivity, subclinical hypothyroidism or isolated hypothyroxinaemia. It is therefore unlikely that maternal thyroid dysfunction at 11-13 weeks has an important contribution to the overall prevalence of spontaneous early preterm delivery. However, the design of our study does not allow conclusions to be drawn as to whether anti-thyroid antibody positivity, subclinical hypothyroidism or isolated hypothyroxinaemia in early pregnancy increase the risk for subsequent spontaneous early preterm delivery.

Chapter 9 Thyroid function in pregnancies with fetal aneuploidies

ABSTRACT

Objective: To examine the association between maternal serum levels of thyroid stimulating hormone (TSH) and free ß human chorionic gonadotrophin (free ß-hCG), in trisomy 21, trisomy 18 and euploid pregnancies at 11-13 weeks and investigate the potential value of TSH in first-trimester screening for aneuploidies.

Methods: Maternal serum TSH and free ß-hCG levels at 11-13 weeks in 25 trisomy 21 and 25 trisomy 18 pregnancies were compared with levels in 3,592 unaffected pregnancies. Only women with no history of thyroid disease and negative for antithyroid antibodies were included.

Results: Serum TSH in the trisomy 21 pregnancies was lower [0.76 multiples of the normal median (MoM), interquartile range (IQR) 0.46-1.09 MoM] and in trisomy 18 it was higher (1.25 MoM, IQR 0.88-1.98 MoM) than in unaffected pregnancies (1.01 MoM IQR 0.61-1.51 MoM). There were significant associations between TSH and free ß-hCG in the unaffected pregnancies (r=-0.214, p<0.0001), but not in those with trisomy 21 (r=-0.157, p=0.452) or trisomy 18 (r=-0.176, p=0.401).

Conclusions: hCG rather than TSH may be the primary thyrotropic factor in early pregnancy. Measurement of TSH does not improve the performance of screening for trisomies 21 and 18 provided by nuchal translucency, free ß-hCG and pregnancy associated plasma protein-A.

This chapter is based on: Ashoor G, Maiz N, Cuckle H, Jawdat F and Nicolaides KH (2011) Maternal thyroid function at 11-13 weeks of gestation in fetal trisomies 21 and 18. *Prenat Diagn*, 31:33-7.

9.1 INTRODUCTION

9.1.1 Background

In early pregnancy there is an inverse association between maternal serum levels of human chorionic gonadotrophin (hCG) and thyroid stimulating hormone (TSH) (see Chapter 1.5). In pregnancies with fetal trisomy 21 the maternal serum concentration of free ß-hCG at 11-13 weeks' gestation is on average twice as high as in euploid pregnancies, whereas in trisomy 18 the levels are one fifth of normal (Macri *et al,* 1990; Spencer *et al.,* 1999; Tul *et al.,* 1999; Kagan *et al.,* 2008a). It is therefore anticipated than in aneuploid pregnancies the maternal serum concentration of TSH would be altered. However, a case control study of pregnancies at 9-11 weeks' gestation reported that there was no significant difference between trisomy 21 and euploid pregnancies in either hCG or TSH (Weinans *et al.,* 2001). However, in this study no corrections were made for maternal characteristics and gestational age that are known to affect the measured concentrations of hCG and TSH.

9.12 Objective

The aim of this study is to examine further the association between maternal serum levels of TSH and hCG in trisomy 21, trisomy 18 and euploid pregnancies at 11-13 weeks, assess any differences in free thyroxine (FT4) and free triiodothyronine (FT3) between the three groups and investigate the potential value of TSH in first-trimester screening for aneuploidies.

9.2 PATIENTS AND METHODS

The study design and overall study population are described in Chapter 2.

In this study we retrospectively examined maternal thyroid function at 11-13 weeks in 30 pregnancies with fetal trisomy 21, 25 with fetal trisomy 18 and 2 with paternally derived triploidy. The diagnosis of aneuloidy was made by chorionic villus sampling

after first-trimester combined screening. In 5 of the trisomy 21 cases the maternal serum concentration of anti-TPO and/or anti-Tg was more than 60 U/mL and these cases were excluded from further analysis because their values were above the manufacturer's reference limit.

The values of FT3, FT4 and TSH in the 25 cases of trisomy 21, 25 cases of trisomy 18 and 2 with triploidy were compared to the results of 3,592 antithyroid antibody-negative singleton pregnancies with no history of thyroid disease, which resulted in live birth after 34 weeks of phenotypically normal neonates with birth weight above the 5th centile (Chapter 3).

Sample analysis

The maternal serum concentrations of FT3, FT4 and TSH were measured by immunoassay as previously described in Chaper 2.

Statistical analysis

The characteristics of the trisomy 21, trisomy 18 and unaffected groups were compared by one-way ANOVA test, followed by Bonferroni post-hoc test if equal variances or Tamhane post-hoc test if unequal variances, for continuous variables and Fisher's exact test or Chi-square test followed by Bonferroni post-hoc test for categorical variables.

The measured concentrations of FT3, FT4 and TSH were converted to MoMs corrected for gestational age, maternal age, racial origin and body mass index (Chapter 3). Similarly, the measured concentration of maternal serum free ß-hCG was MoMs corrected for fetal CRL, maternal weight, smoking status, racial origin, parity and method of conception (Kagan *et al.*, 2008b). Comparison of TSH MoM, FT3 MoM and FT4 MoM between aneuploid and unaffected groups was by one-way ANOVA test, followed by Bonferroni post-hoc test if equal variances or Tamhane post-hoc test if unequal variances. Pearson correlation was used to determine the significance of the

inter-relations between serum square root (√) TSH MoM, Log_{10} FT3 MoM, Log_{10} FT4 MoM Log_{10} PAPP-A MoM and Log_{10} free ß-hCG MoM.

The added value of including thyroid function markers in aneuploidy screening was estimated by standard modeling techniques (Royston and Thompson, 1992). The model parameters - means, standard deviations and correlation coefficients and maternal age distribution - were derived directly from the study data. Trisomy 21 detection rates were estimated for fixed 3% and 5% false-positive rates; trisomy 18 rates for 0.5% and 1%.

The statistical software packages PASW statistics 18.0 (SPSS Inc.,Chicago, IL) was used for the data analyses.

9.3 RESULTS

The maternal characteristics and results of first-trimester combined screening for aneuploidies in trisomy 21, trisomy 18 and unaffected pregnancies are compared in Table 9.1. In both the trisomy 21 and trisomy 18 groups, compared to the unaffected group, maternal age and fetal NT thickness were higher and serum PAPP-A was lower. Serum free ß-hCG in trisomy 21 was increased and in trisomy 18 it was decreased.

Serum TSH in the trisomy 21 pregnancies was lower and in trisomy 18 it was higher than in unaffected pregnancies (Table 9.2). There were no significant differences between the groups in serum FT4 but in trisomy 18 pregnancies FT3 was significantly reduced. In the unaffected pregnancies, but not in those with trisomy 21 or trisomy 18, there were significant intercorrelations between TSH, free ß-hCG, FT3 and FT4 (Table 9.3).

None of the thyroid function markers increased the model predicted detection rates for trisomy 21 compared with the existing first trimester screening protocol by more than

Table 9.1. Maternal characteristics and results of first-trimester combined screening for aneuploidies in trisomy 21, trisomy 18 and unaffected pregnancies.

Variables	Unaffected (n=3,592)	Trisomy 21 (n=25)	Trisomy 18 (n=25)
Maternal age in yrs (median, IQR)	32.2 (27.9-36.0)	38.7 (35.2-41.8)**	37.9 (33.6-40.4)**
Body mass index in Kg/m^2, median (IQR)	24.7 (22.2-27.9)	24.8 (22.5-28.5)	25.1 (21.6-29.2)
Racial origin			
Caucasian, n (%)	2,543 (70.8)	20 (80.0)	18 (72.0)
African, n (%)	708 (19.7)	4 (16.0)	4 (16.0)
South Asian, n (%)	148 (4.1)	0	2 (8.0)
East Asian, n (%)	57 (1.6)	1 (4.0)	0
Mixed, n (%)	136 (3.8)	0	1 (4.0)
Parity			
Nulliparous, n (%)	1684 (46.9)	9 (36.0)	9 (36.0)
Parous	1908 (53.1)	16 (64.0)	16 (64.0)
Cigarette smoker, n (%)	322 (9.0)	3 (12.0)	2 (8.0)
Conception by ovulation drugs, n (%)	101 (2.8)	4 (16.0)**	6 (24.0)**
Fetal crown-rump length in mm (median, IQR)	63.5 (59.0-68.7)	64.2 (58.0-70.1)	57.3 (51.0-61.8)**
Fetal NT thickness in mm (median, IQR)	1.8 (1.5-2.0)	3.7 (2.8-5.0)**	5.4 (2.0-6.4)**
Delta NT thickness in mm (median, IQR)	0.09 (-0.11 to 0.29)	2.13 (1.30 to 3.23)**	3.88 (0.39 to 5.04)**
Serum free ß-hCG MoM (median, IQR)	0.96 (0.66-1.50)	1.87 (1.46-3.35)**	0.23 (0.17-0.34)**
Serum PAPP-A MoM (median, IQR)	1.00 (0.69-1.42)	0.54 (0.33-0.69)**	0.26 (0.16-0.38)**

NT, Nuchal translucency; hCG, human chorionic gonadotrophin; PAPP-A, pregnancy associated plasma protein-A;

Comparison between each aneuploid group and the unaffected pregnancies was by Chi square or Fisher exact test for categorical variables with post-hoc Bonferroni correction and ANOVA test for categorical both with post-hoc Bonferroni test for maternal age, BMI, and CRL and post-hoc Tamhane test for fetal NT, delta NT and serum free ß-hCG MoM and PAPP-A.
*p<0.05, ** p<0.001

0.8%. The increase in detection for trisomy 18 was at most 0.2%. The Mahalinobis distance for the decrease in TSH MoM values in trisomy 21 was 0.46 and for the increase in trisomy 18 was 0.37.

In the two cases of paternally derived fetal triploidy the gestational age was 13 weeks and the fetal CRL was 63.5 mm and 73.7 mm, respectively. The maternal serum free ß-hCG was 7.51 and 6.77 MoM, the levels of TSH were undetectable and both FT3 (1.63 and 3.98 MoM) and FT4 (1.71 and 3.41 MoM) were increased.

Table 9.2. Maternal thyroid function in trisomy 21, trisomy 18 and unaffected pregnancies.

	Unaffected (n=3,592)	**Trisomy 21 (n=25)**	**Trisomy 18 (n=25)**
Thyroid stimulating hormone			
MoM (median, IQR)	1.01 (0.61-1.51)	0.76 (0.46-1.09)*	1.25 (0.88-1.98)*
m IU/L (median, IQR)	1.10 (0.67-1.67)	0.85 (0.49-1.22)*	1.46 (1.03-2.11)*
Free thyroxine			
MoM (median, IQR)	0.99 (0.91-1.09)	1.04 (0.91-1.23)	0.97 (0.83-1.03)
Pmol/L (median, IQR)	14.9 (13.6-16.3)	15.4 (13.5-18.2)	14.4 (12.2-15.4)
Free triiodothyronine			
MoM (median, IQR)	0.99 (0.93-1.06)	1.02 (0.97-1.18)	0.95 (0.91-1.00)*
Pmol/L (median, IQR)	4.6 (4.4-5.0)	4.7 (4.5-5.5)	4.4 (4.2-4.8)*

Comparison between each aneuploid group and the unaffected pregnancies was by ANOVA test for categorical both with post-hoc Bonferroni test for free thyroxine and post-hoc Tamhane test for Thyroid stimulating hormone and free triiodothyronine.
*p<0.05

Table 9.3. Pearson correlation between square root (√) TSH MoM, Log FT4 MoM and Log FT3 MoM and Log free β-hCG MoM in trisomy 21, trisomy 18 and unaffected pregnancies.

	Unaffected (n=3,592)		**Trisomy 21 (n=25)**		**Trisomy 18 (n=25)**	
Correlations	**r**	**p**	**r**	**P**	**r**	**p**
√ TSH MoM with Log FT4 MoM	-0.245	<0.0001	0.106	0.613	-0.111	0.596
√ TSH MoM with Log FT3 MoM	-0.182	<0.0001	-0.307	0.135	0.238	0.251
Log FT4 MoM with Log FT3 MoM	0.476	<0.0001	0.376	0.064	0.359	0.078
Log β-hCG MoM with √ TSH MoM	-0.214	<0.0001	-0.157	0.452	-0.176	0.401
Log β-hCG MoM with Log FT4 MoM	0.124	<0.0001	0.252	0.223	-0.015	0.944
Log β-hCG MoM with Log FT3 MoM	0.104	<0.0001	0.205	0.325	-0.093	0.658

9.4 DISCUSSION

This study has demonstrated that in euploid pregnancies there is a weak inverse association between free ß-hCG MoM and TSH MoM. In trisomy 21 pregnancies free ß-hCG is increased and TSH is decreased and in trisomy 18 pregnancies free ß-hCG is decreased and TSH is increased.

In first trimester screening for aneuploidy the observed decrease in TSH MoM values in trisomy 21 and the increase in trisomy 18 pregnancies had only modest discriminatory value, which was about half that of AFP which is the weakest current marker. Moreover, the negative correlation between free ß-hCG and TSH means that the use of both markers in screening will effectively decrease the Mahalinobis distances. This is reflected in the lack of any model predicted additional detection if TSH was added to current first trimester screening protocols.

The finding of an inverse association between free ß-hCG MoM and TSH MoM is compatible with the known thyrotropic properties of hCG (Braunstein and Hershman, 1976; Pekonen *et al.,* 1988; Glinoer *et al.,* 1990; Ballabio *et al.,* 1991). In pregnancy there is a mirror image between TSH and hCG levels with high hCG and low TSH at 8-14 weeks and subsequent increase in TSH during the second and third trimesters coinciding with the decline in hCG (Glinoer *et al.,* 1990). Pregnancy is associated with an approximate 50% increase in demand for thyroid hormones which is apparent within the first 16 weeks of gestation and is mainly attributed to the estrogen-driven doubling in thyroxine-binding globulin concentrations (Glinoer *et al.,* 1990; Alexander *et al.,* 2004). Consequently, the increased demands for maternal thyroid hormones of early pregnancy are under the influence of the placenta rather than the maternal pituitary gland. It could even be postulated that the otherwise unknown role of hCG resides in its thyrotropic properties at this critical stage of development when the fetal requirements for thyroid hormones are entirely dependent on the mother. Although the fetal thyroid gland begins to produce thyroid hormones from as early as 11 weeks of gestation (Shepard, 1967) functional maturation of the fetal pituitary-thyroid axis occurs only during the second half of pregnancy (Thorpe-Beeston *et al.,* 1991a, 1991b).

In this study we measured the screening marker free ß-hCG which is not a hormonally active molecule but its levels reflect those of the hormonally active intact hCG molecule. This interrelation between hCG and TSH maintains normal FT3 and FT4 levels and this is reflected in trisomy 21 pregnancies where despite doubling in free ß-hCG levels, FT3 and FT4 were not significantly different from euploid pregnancies.

However, in cases of very high levels in free ß-hCG, as observed in our two cases of androgenic triploidy, serum FT3 and FT4 were increased with complete suppression of TSH production. The opposite was true in the case of trisomy 18 where very low levels of free ß-hCG were accompanied by an increase in TSH but the level of FT3 was reduced. Although the level of FT4 was also lower than in euploid pregnancies the difference was not significant. These data provide further support to the hypothesis that hCG rather than TSH may be the primary thyrotropic factor in early pregnancy. Although serum TSH is altered in pregnancies with fetal trisomies 21 and 18 this measurement does not improve the performance of screening for these aneuploidies provided by nuchal translucency, free ß-hCG and pregnancy associated plasma protein-A.

9.5 CONCLUSIONS

The data of this study provide further support to the hypothesis that hCG rather than TSH may be the primary thyrotropic factor in early pregnancy. Although serum TSH is altered in pregnancies with fetal trisomies 21 and 18 this measurement does not improve the performance of screening for these aneuploidies provided by nuchal translucency, free ß-hCG and pregnancy associated plasma protein-A.

Chapter 10 Thyroid function in twin pregnancies

ABSTRACT

Objective: To establish reference ranges of maternal serum thyroid stimulating hormone (TSH), free thyroxine (FT4) and free triiodothyronine (FT3) at 11-13 weeks' gestation in twin pregnancy.

Methods: This was a case series of 177 dichorionic and 58 monochorionic twin pregnancies with normal outcome and 19 monochorionic pregnancies complicated by severe twin-to-twin transfusion syndrome (TTTS). Maternal serum concentrations of TSH, FT3, FT4, anti-thyroperoxidase and anti-thyroglobulin antibodies were measured at 11-13 weeks' gestation. The measured TSH, FT3 and FT4 were converted to multiple of median (MoM) of normal singleton pregnancy and MoM values in the different groups were compared.

Results: In the antithyroid antibody negative twin pregnancies with normal outcome, compared to singletons, serum TSH MoM was lower (median 0.62, IQR 0.16-1.18 vs. 1.01, IQR 0.61-1.51; P<0.0001), FT3 MoM and FT4 MoM were not significantly different (FT3: median 0.99, IQR 0.90-1.06 vs. 0.99, IQR 0.94-1.06; p=0.132; FT4: median 0.98, IQR 0.91-1.08 vs. 0.99, IQR 0.91-1.09; P=0.975) and free ß-hCG MoM was higher (median 1.91, IQR 1.33-2.59 vs. 0.98, IQR 0.66-1.50; P<0.0001). In the antibody-positive group (n=37), compared to the negative group (n=198), the median TSH was higher but FT4, FT3 and free ß-hCG were not significantly different. In the TTTS group, compared to normal twin pregnancies, TSH, FT4, FT3 and free ß-hCG were not significantly different.

Conclusion: In twins, compared to singleton pregnancies, TSH is lower but FT3 and FT4 are not significantly different.

This chapter is based on: Ashoor G, Muto O, Poon LCY, Muhaisen M and Nicolaides KH (2013) Maternal thyroid function at 11-13 weeks' gestation in twin pregnancy. *Thyroid,* in press

10.1 INTRODUCTION

10.1.1 Background

A study in 132 twin pregnancies, compared to 13,599 singletons, reported that serum TSH is lower (Dashe *et al.,* 2005). This has been attributed to the higher levels of human chorionic gonadotrophin (hCG) in twins, which stimulates thyroid hormone secretion with consequent suppression of TSH.

10.1.2 Objective

The aim of this study is to establish a normal range of serum TSH, FT3 and FT4 in dichorionic and monochorionic twins at 11-13 weeks' gestation and compare the values to singleton pregnancies.

10.2 PATIENTS AND METHODS

The study design and overall study population are described in Chapter 2.

This was a case series of Dichorionic and monochorionic twin pregnancies attending for their routine first hospital visit in pregnancy held at 11-13 weeks' gestation (Sepulveda *et al.,* 1996). The inclusion criteria were: twin pregnancies with live fetuses at 11-13 weeks, delivery of live births at or after 33 weeks' gestation.

We excluded cases with: maternal history of hypothyroidism or hyperthyroidism, fetal abnormalities, preeclampsia and small for gestational age neonates with birth weight below the 5th percentile of our normal range for gestation (Poon *et al.,* 2011b). These criteria were met by 235 cases with available serum for analysis (normal group). Additionally, we examined 19 cases that developed severe twin-twin-transfusion syndrome (TTTS) requiring endoscopic laser surgery (Ville *et al.,* 1998). None of the women suffered from hyperemesis gravidarum at the time of testing.

Sample analysis

The maternal serum concentrations of FT3, FT4 and TSH were measured by immunoassay as previously described in Chaper 2.

Statistical analysis

The characteristics of the dichorionic and monochorionic twins were compared by the Mann-Whitney-U test for continuous variables and Fisher's exact test or chi-square test for categorical variables.

The measured serum TSH, FT3 and FT4 in the twins with normal outcome were converted to MoM of normal singleton pregnancy, corrected for gestational age, maternal age, BMI and racial origin as previously described (Chapter 3). The limitation with this statistical approach is the assumption that the effects of maternal characteristics such as age, race and BMI on thyroid functions are the same in twins as in singleton pregnancy. In the antibody-negative group, regression analysis was used to determine if twinning was a significant predictor of square-root TSH MoM, $\log_{10}$ FT3 MoM and $\log_{10}$ FT4 MoM. These transformations of square-root for TSH and $\log_{10}$ for FT3 and FT4 were used to achieve Gaussian normality of the distributions (Chapter 3). The observed values of TSH, FT3 and FT4 were then expressed as MoM for normal twin pregnancy. Similarly MoM values were also calculated for the TTTS group.

Regression analysis was used to determine the significance of the inter-relations between serum TSH, FT3, FT4 and free β-hCG. Comparison of TSH MoM, FT3 MoM and FT4 MoM between the antibody-positive and antibody-negative groups was done by the Kruskal-Wallis test with post hoc Bonferroni correction (critical statistical significance $p<0.0167$). Similarly, the normal outcome group was compared with the TTTS group.

The proportion of cases with serum TSH above the 97.5th percentile, serum FT3 and FT4 below the 2.5th percentile in the antibody-positive and -negative groups were compared using the chi-square test with post hoc Bonferroni correction.

The statistical software package SPSS 20.0 (SPSS Inc., Chicago, Ill., USA) was used for data analyses.

10.3 RESULTS

In the 235 twin pregnancies with normal outcome there were 177 (75.3%) with dichorionic and 58 (24.7%) with monochorionic twins (Table 10.1).

Table 10.1. Comparison of maternal characteristics in dichorionic and monochorionic twin pregnancies with normal outcome.

Maternal characteristics	Dichorionic (n=177)	Monochorionic (n=58)	P-value
Median maternal age, years (IQR)	33.7 (29.7-36.8)	33.6 (28.9-36.7)	0.801
Median body mass index, kg/m^2 (IQR)	25.3 (23.0-29.3)	24.4 (21.9-28.3)	0.178
Median gestational age, week (IQR)	13.0 (12.6-13.5)	13.0 (12.6-13.5)	0.912
Racial origin			
Caucasian, n (%)	136 (76.8)	45 (77.6)	1.000
African, n (%)	30 (16.9)	5 (8.6)	0.141
South Asian, n (%)	6 (3.4)	5 (8.6)	0.145
East Asian, n (%)	1 (0.6)	0 (0.0)	1.000
Mixed, n (%)	4 (2.3)	3 (5.2)	0.368
Conception			
Spontaneous, n (%)	88 (49.7)	30 (51.7)	0.880
Assisted, n (%)	89 (51.3)	28 (48.3)	0.880
Antibody negative, n (%)	152 (85.9)	46 (79.3)	0.298
Antibody positive, n (%)	25 (14.1)	12 (20.7)	0.298

IQR = Interquartile range. * Comparisons by chi-square test for categorical variables and by Mann-Whitney U test for continuous variables (P < 0.05).

In 198 (84.3%) of the 235 pregnancies the serum concentration of anti-TPO and anti-Tg was <60 U/mL and in 37 (15.7%) the concentration of one or both antibodies was ≥60 U/mL. In 17 (7.2%) both antibodies were positive, in 12 (5.1%) only anti-TPO was positive and in 8 (3.4%) only anti-Tg was positive. The prevalence of antibody positivity was not significantly different between dichorionic (25 of 177, 14.1%) and monochorionic twins (12 of 58, 20.7%; P=0.298).

Antithyroid antibody negative twin pregnancies with normal outcome

In the antithyroid antibody negative twin pregnancies with normal outcome, compared to singletons, serum TSH MoM was lower (median 0.62, IQR 0.16-1.18 vs. 1.01, IQR 0.61-1.51; P<0.0001; Figures 10.1 and 10.2), FT3 MoM and FT4 MoM were not significantly different (FT3: median 0.99, IQR 0.90-1.06 vs. 0.99, IQR 0.94-1.06; p=0.132; FT4: median 0.98, IQR 0.91-1.08 vs. 0.99, IQR 0.91-1.09; P=0.975; Figure 10.2) and free ß-hCG MoM was higher (median 1.91, IQR 1.33-2.59 vs. 0.98, IQR 0.66-1.50; P<0.0001; Figure 10.2).

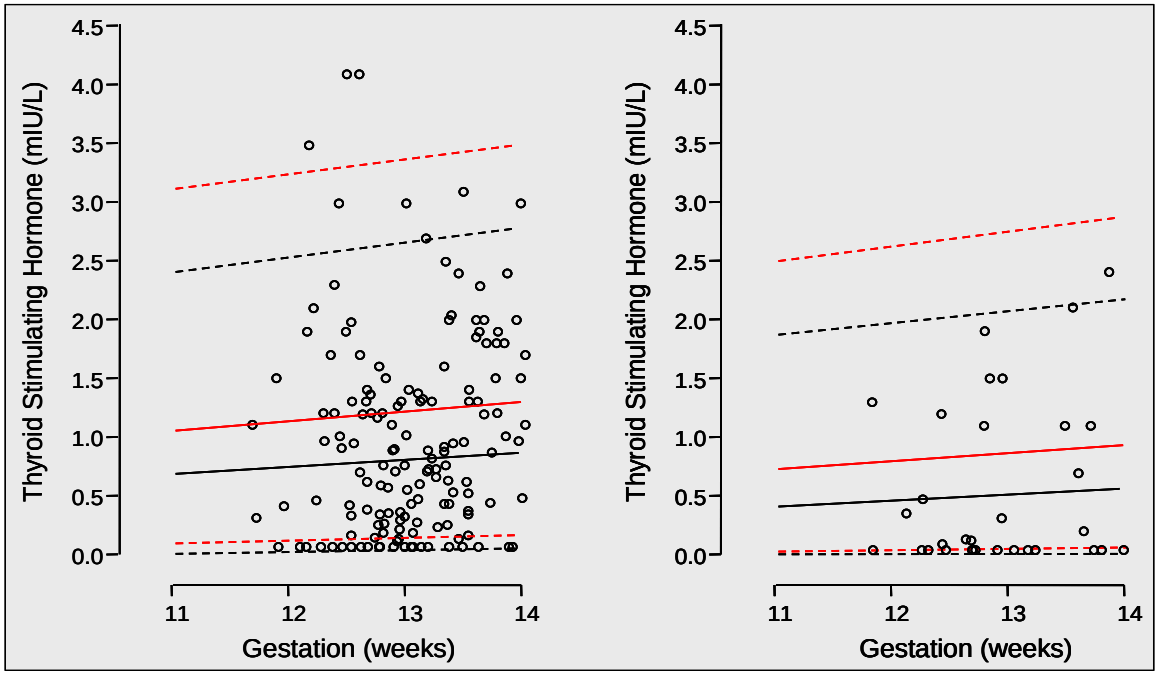

Figure 10.1. Maternal serum thyroid stimulating hormone concentration of twin pregnancies in Caucasian (left) and African (right) women plotted on the normal ranges (2.5th, 50th, 97.5th percentile) of singleton pregnancies (red lines) and twin pregnancies (black lines).

Regression analysis demonstrated that twinning, but not chorionicity (TSH: P=0.722; FT3: P=0.578; FT4: P=0.911), had significant contribution to the level of TSH but not FT3 and FT4:

Expected square-root TSH MoM = 0.999933 (s.e. 0.006139) + [-0.211140 (s.e. 0.026860) if twin pregnancy]; R^2=0.016, P<0.0001

Expected $\log_{10}$ free T3 MoM = 0.000000 (s.e. 0.000783) + [-0.001131 (s.e. 0.003426) if twin pregnancy]; R^2=0.000, P=0.741

Expected $\log_{10}$ free T4 MoM = 0.000000 (s.e. 0.001076) + [0.003856 (s.e. 0.004708) if twin pregnancy]; R^2=0.000, P=0.413

The observed values of TSH, FT3 and FT4 were then expressed as MoM for normal twin pregnancy. The 50th, 95th, 97.5th, 5th and 2.5th percentiles of serum TSH, FT3 and FT4 for twin pregnancies are shown in Table 10.2.

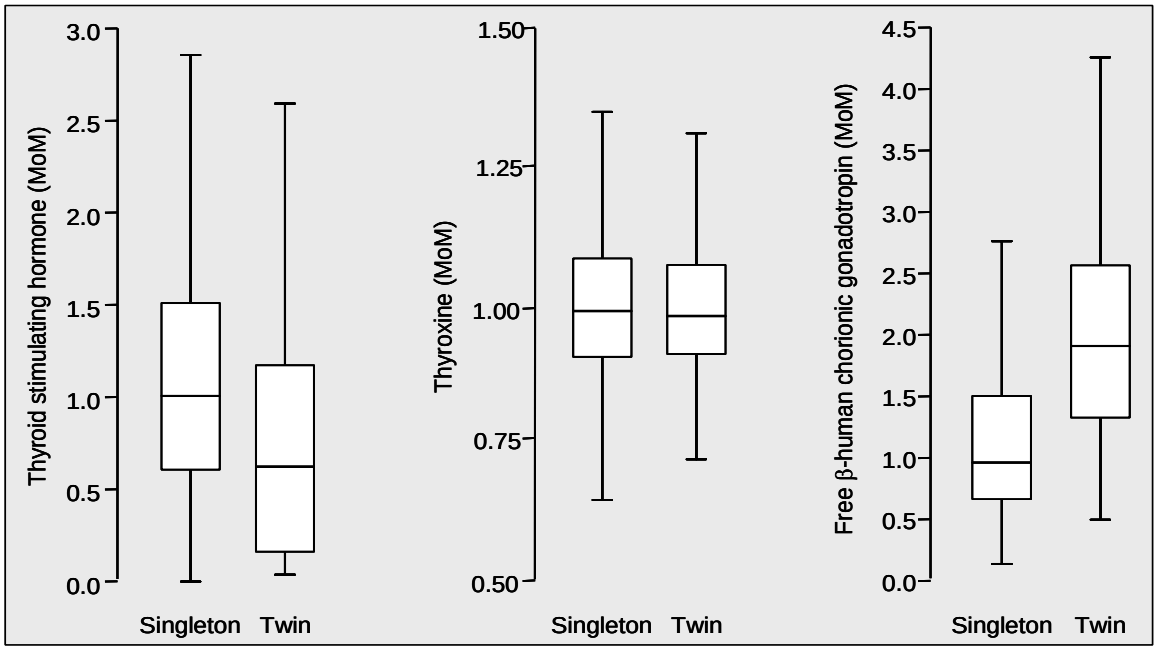

Figure 10.2. Box-whisker plots of the multiples of median (MoM) thyroid stimulating hormone and free thyroxine in singleton and twin pregnancies.

In dichorionic twins, compared to monochorionic twins, there were no significant differences in TSH MoM (median 0.98, IQR 0.28-1.29 vs. 1.13, IQR 0.23-2.22; P=0.856), FT3 MoM (median 0.99, IQR 0.90-1.03 vs. 1.00, IQR 0.96-1.10; P=0.052), FT4 MoM (median 0.98, IQR 0.91-1.08 vs. 1.01, IQR 0.94-1.06; P=0.537) or free ß-hCG MoM (median 0.98, IQR 0.70-1.29 vs. 1.13, IQR 0.68-1.63; P=0.299).

Table 10.2. Maternal serum concentration of thyroid stimulating hormone, free thyroxine and free triiodothyronine at 11-13 weeks in normal twin pregnancy.

Race	Gestation (wees)	Body mass Index	Thyroid stimulating hormone (mIU/L)					Age Years	Free thyroxine (pmol/L)					Age years	Free triiodothyronine (pmol/L)				
			2.5th	5th	50th	95th	97.5th		2.5th	5th	50th	95th	97.5th		2.5th	5th	50th	95th	97.5th
Caucasian	11	<25	0.01	0.04	0.66	2.01	2.35	<30	11.64	12.21	15.64	20.04	21.02	<30	3.90	4.04	4.82	5.75	5.95
								>30	11.53	12.09	15.49	19.85	20.82	>30	3.82	3.95	4.72	5.63	5.82
		>25	0.01	0.05	0.69	2.05	2.40	<30	11.44	11.99	15.37	19.69	20.65	<30	3.96	4.10	4.89	5.84	6.04
								>30	11.33	11.88	15.22	19.51	20.46	>30	3.88	4.01	4.79	5.72	5.92
	12	<25	0.02	0.06	0.72	2.11	2.46	<30	11.47	12.03	15.41	19.75	20.71	<30	3.86	4.00	4.77	5.69	5.89
								>30	11.36	11.92	15.27	19.56	20.52	>30	3.78	3.91	4.67	5.57	5.77
		>25	0.02	0.07	0.74	2.16	2.51	<30	11.27	11.82	15.14	19.41	20.35	<30	3.92	4.06	4.85	5.78	5.98
								>30	11.16	11.71	15.00	19.22	20.16	>30	3.84	3.97	4.74	5.66	5.86
	13	<25	0.03	0.08	0.78	2.21	2.57	<30	11.30	11.85	15.19	19.46	20.41	<30	3.82	3.96	4.72	5.64	5.83
								>30	11.20	11.74	15.05	19.28	20.22	>30	3.74	3.87	4.62	5.52	5.71
		>25	0.03	0.09	0.81	2.26	2.62	<30	11.11	11.65	14.92	19.12	20.05	<30	3.88	4.02	4.80	5.73	5.92
								>30	11.00	11.54	14.78	18.94	19.86	>30	3.80	3.93	4.70	5.60	5.80
African	11	<25	0.00	0.00	0.39	1.52	1.82	<30	11.36	11.91	15.26	19.55	20.50	<30	3.82	3.95	4.72	5.63	5.83
								>30	11.25	11.79	15.11	19.37	20.31	>30	3.74	3.87	4.62	5.51	5.70
		>25	0.00	0.00	0.42	1.56	1.87	<30	11.16	11.70	14.99	19.21	20.14	<30	3.88	4.01	4.79	5.72	5.92
								>30	11.05	11.59	14.85	19.03	19.95	>30	3.80	3.93	4.69	5.60	5.79
	12	<25	0.00	0.00	0.44	1.61	1.92	<30	11.19	11.73	15.04	19.27	20.20	<30	3.78	3.91	4.67	5.58	5.77
								>30	11.08	11.62	14.89	19.08	20.01	>30	3.70	3.83	4.57	5.46	5.65
		>25	0.00	0.01	0.46	1.65	1.96	<30	10.99	11.53	14.77	18.93	19.85	<30	3.84	3.98	4.75	5.66	5.86
								>30	10.89	11.42	14.63	18.75	19.66	>30	3.76	3.89	4.64	5.54	5.74
	13	<25	0.00	0.01	0.49	1.70	2.02	<30	11.03	11.56	14.82	18.98	19.91	<30	3.75	3.87	4.62	5.52	5.71
								>30	10.92	11.45	14.67	18.80	19.72	>30	3.67	3.79	4.53	5.40	5.59
		>25	0.00	0.01	0.51	1.74	2.06	<30	10.83	11.36	14.56	18.65	19.56	<30	3.80	3.94	4.70	5.61	5.80
								>30	10.73	11.25	14.42	18.47	19.37	>30	3.72	3.85	4.60	5.49	5.68

There was a significant correlation between $\log_{10}$ TSH MoM and $\log_{10}$ FT4 MoM (r=-0.487, P<0.0001) and $\log_{10}$ FT3 MoM (r=-0.502, P<0.0001) and $\log_{10}$ free β-hCG MoM (r=-0.280, P<0.0001); and between $\log_{10}$ FT4 MoM and $\log_{10}$ FT3 MoM (r=0.633, P<0.0001) and $\log_{10}$ free β-hCG MoM (r=0.199, P=0.005); and also between $\log_{10}$ FT3 MoM and $\log_{10}$ free β-hCG MoM (r=0.157, P=0.027).

Antithyroid antibody positive twin pregnancies with normal outcome

In the cases where both anti-TPO and anti-Tg were positive, compared to the antibody-negative group, the median TSH was higher but FT4, FT3 and free ß-hCG were not significantly different (Table 10.3). Serum TSH was above the 97.5th percentile in 2.0% of the antibody-negative group and this increased to 29.4% (5 of 17) in the group with both anti-TPO and anti-Tg positivity. There was no significant difference in the proportion of FT3 and FT4 below the 2.5th percentile between the antibody-negative and -positive groups (Table 10.3).

Table 10.3. Comparison of the antibody-positive and antibody-negative groups for median thyroid stimulating hormone (TSH), free thyroxine (FT4), free triiodothyronine (FT3) and free β-human chorionic gonadotropin (β-hCG) and proportion of cases with TSH above the 97.5th percentile and FT4 and FT3 below the 2.5th percentile of the respective reference range.

Thyroid function	Antibody negative (n=198)	Antibody positive		
		anti-TPO only (n=12)	anti-Tg only (n=8)	both (n=17)
Thyroid stimulating hormone				
Median MoM	1.00	2.00	0.64	2.73*
>97.5th percentile, n (%)	4 (2.0)	2 (16.7)	0 (0.0)	5 (29.4)*
Free thyroxine				
Median MoM	0.98	1.02	0.99	0.97
<2.5th percentile, n (%)	4 (2.0)	2 (16.7)	0 (0.0)	2 (11.8)
Free triiodothyronine				
Median MoM	0.99	0.94	0.91	0.88
<2.5th percentile, n (%)	4 (2.0)	2 (16.7)	1 (12.5)	2 (11.8)
Free β-hCG median MoM	1.00	1.39	1.19	1.13

Comparisons between each antibody-positive group with the antibody-negative group by chi-square test with post hoc Bonferroni correction for categorical variables and by Kruskal-Wallis with post hoc Bonferroni correction for continuous variables. * P < 0.0167.

Pregnancies complicated by twin-to-twin transfusion syndrome

In the TTTS group, 17 (89.5%) of the 19 pregnancies were antithyroid antibody negative and in this group, compared to the antibody negative normal outcome twins, there was no significant difference in TSH MoM (1.38, IQR 0.52-2.05 vs 1.00, IQR 0.26-1.36, P=0.424), FT3 MoM (0.97, IQR 0.86-1.09 vs 0.99, IQR 0.90-1.06, P=0.246), FT4 MoM (0.94, IQR 0.90-1.16 vs 0.98, IQR 0.91-1.08, P=0.773), or free ß-hCG MoM (0.95, IQR 0.51-2.22 vs 1.00, IQR 0.69-1.36, P=0.997).

10.4 DISCUSSION

This study has demonstrated that in normal twin pregnancies at 11-13 weeks' gestation, compared to singletons, maternal serum FT4 is not significantly different but TSH is about 40% lower. The most likely explanation for the low TSH is the high level of free ß-hCG, which is twice as high as in singletons. The levels of serum TSH, FT3 and FT4 were similar in dichorionic and monochorionic twins, with or without TTTS, and there were no significant differences between the three groups in serum free ß-hCG.

In establishing reference ranges of thyroid function in twins we excluded pregnancies complicated by miscarriage or fetal death, fetal growth restriction, preeclampsia and preterm delivery because of the reported association between these pregnancy complications and clinical or subclinical hypothyroidism (Leung *et al.,* 1993; Allan *et al.,* 2000; Casey *et al.,* 2005). We also excluded pregnancies with known thyroid disease and those with anti-thyroid antibodies. In our population about 12% of pregnancies had detectable anti-TPO antibodies and 11% had anti-Tg antibodies, which are similar to the respective prevalence of 10% and 14% in our singleton pregnancies (Chapter 3). In the antibody-positive group when both antibodies were positive, compared to the antibody negative group, there were higher median TSH and percentage of cases with TSH values above the 97.5th percentile, whereas serum FT3, FT4 and β-hCG were not significantly different. A previous study reported that the majority of antibody-

positive women with subclinical hypothyroidism during pregnancy will develop clinical hypothyroidism within the subsequent 10 years (Haddow *et al.,* 1999). Consequently, in establishing normal ranges of thyroid function it is necessary to exclude antibody positive patients.

The measured serum TSH, FT3 and FT4 in twin pregnancies were converted to MoM of normal singleton pregnancy, corrected for gestational age in weeks, maternal age, BMI and racial origin as previously described (Chapter 3). In women of African racial origin the serum concentration of both TSH and FT4 is lower than in Caucasians, suggesting that the hypothalamo-pituitary-thyroid gland axis in the two racial groups is set at different thresholds. The minimum detectable concentration for TSH is 0.01 mIU/l and in the Caucasian population the 2.5th centile raw values were all detectable. However, clinically most hospitals use the 5th or even the 10th centile as cut-offs. In the African population the 2.5th and the 5th centile can be undetectable depending on other maternal characteristics and gestation. This illustrates that more accurate assays will help in diagnosing hyperthyroidism in pregnancy in that group. None of the women in our cohort had undetectable serum TSH levels and this is probably due to the fact that we only examined 32 women of African origin.

The most likely explanation for the finding that in antithyroid antibody negative twins, compared to singletons, maternal serum FT3 and FT4 were not significantly different but TSH was lower due to the increase in ß-hCG. Human chorionic gonadotrophin, which has an identical α-subunit and structurally similar ß-subunit to those of TSH, has thyrotropic properties and in early pregnancy there is an inverse association between maternal serum levels of TSH and hCG (Braunstein *et al.,* 1976; Yoshikawa *et al.,* 1989; Glinoer *et al.,* 1990; Ballabio *et al.,* 1991; Yoshimura and Hershman, 1995; Grun *et al.,* 1997). A study of 3,961 dichorionic and 759 monochorionic twins at 8-13 weeks' gestation reported that the maternal serum free ß-hCG, expressed as MoMs for singleton pregnancies, increased from 1.5 MoM at 8-9 weeks to 2.0 MoM at 12-13 weeks for dichorionic twins and from 1.0 MoM to 2.0 MoM for monochorionic twins (Madsen *et al.,* 2011). Consequently, at the median gestational age of 13 weeks in our

study the levels of free ß-hCG in twins, irrespective of chorionicity, were twice as high as in singletons. Similarly, the levels of free ß-hCG and TSH in monochorionic twins that subsequently developed severe TTTS were similar to those of normal twins.

10.5 CONCLUSIONS

The study established reference ranges of maternal thyroid function in twin pregnancies at 11-13 weeks after appropriate correction for maternal characteristics which affect the measured serum concentrations of TSH, FT3 and FT4. These ranges can be used in clinical practice to help diagnose subclinical and overt hypo- and hyperthyroidism. Given the lower levels of TSH in twin pregnancies it is important to use the twin-specific normal range otherwise there is a risk of over-diagnosis of thyrotoxicosis in twin pregnancies. They can also form the basis for the study of early thyroid function in pathological pregnancies and the investigation of the consequences of overt and subclinical hypothyroidism on twin pregnancy outcome.

Chapter 11 Conclusions and suggestions for future studies

11.1 CONCLUSIONS

The study in Chapter 3 established normal ranges for maternal thyroid function at 11-13 weeks' gestation after adjustment for maternal characteristics which affect the measured serum concentrations of thyroid stimulating hormone (TSH), free triiodothyronine (FT3) and free thryroxin (FT4). In antithyroid antibody negative women serum TSH increased whereas FT3 and FT4 decreased with gestation and all three were lower in Afro-Caribbean than in Caucasian women. Serum FT3 and FT4 decreased but TSH did not change significantly with maternal age, TSH and FT3 increased whereas FT4 decreased with body mass index, TSH decreased whereas FT3 and FT4 increased with serum free ß-hCG. In the antibody positive group, compared to the negative group, median TSH was higher and median FT3 and FT4 were lower.

The study in Chapter 4 assessed thyroid function at 11-13 weeks' gestation in women with known hypothyroidism diagnosed before pregnancy and receiving levothyroxine. On the basis of their individual results about 55% of the patients were biochemically euthyroid with normal serum TSH and normal or high FT4 and FT3. In the remaining 45% at least one of the three biochemical tests was suggestive of persistent hypothyroidism. There was a small group with low FT4 and FT3 and high TSH. In a much larger group serum FT4 was normal or increased but either TSH was high and / or FT3 was low. These findings raise the question as to whether the objective in the treatment of hypothyroidism in pregnancy should be to normalize TSH or FT4 or FT3.

The study in Chapter 5 demonstrated that in pregnancies resulting in miscarriage or fetal death during the second and third trimesters, compared to those with normal outcome, the median maternal serum concentration of TSH at 11-13 weeks' gestation was increased and FT4 was decreased and the incidence of high TSH and low FT4

was increased. Previously undiagnosed hypothyroidism diagnosed at 11-13 weeks' gestation may be a contributing factor to about 5% of subsequent fetal losses. The study also showed that there were no significant differences between the fetal loss and normal groups in the incidence of antithyroid antibody positivity, contradicting a previous hypothesis that antithyroid antibodies exert a direct toxic effect on the pregnancy leading to fetal loss.

The study in Chapter 6 demonstrated an association between impaired maternal thyroid function at 11-13 weeks' gestation and subsequent development of late but not early preeclampsia (PE). High serum TSH was observed in 5 times as many pregnancies with late-PE compared with those who did not develop PE. This association of hypothyroidism and PE is independent of autoimmune mechanisms because the prevalence of antithyroid antibodies was not higher in the PE than in the non-PE group. The association between hypothyroidism and late-PE may be mediated by the role of thyroid hormones in glucose homeostasis and in the synthesis, metabolism and mobilization of lipids. Hypothyroidism may also play a direct role in causing pregnancy hypertension because thyroid hormones act directly on peripheral arterioles to cause dilation. The study also showed that measurement of maternal serum TSH can improve the prediction of late-PE provided by a combination of factors in the maternal history and the measurements of mean arterial pressure and uterine artery pulsatility index.

The study in Chapter 7 demonstrated that in pregnancies delivering small for gestational age (SGA) neonates, maternal thyroid function at 11-13 weeks' gestation was not significantly different from those delivering appropriately grown neonates and there was no evidence that in the SGA group the incidence of impaired thyroid function is increased. Consequently, the results of *in vitro* studies concerning the role of thyroid hormones on trophoblast proliferation and invasion (Barber *et al.,* 2005; Oki *et al.,* 2004) may not be clinically relevant and thyroid function does not have a significant contribution to the prevalence of SGA neonates.

The study in Chapter 8 demonstrated that there was no significant difference between pregnancies ending in spontaneous early preterm delivery and those delivering at term in the prevalence of anti-thyroid antibody positivity, subclinical hypothyroidism or isolated hypothyroxinaemia. It is therefore unlikely that maternal thyroid dysfunction at 11-13 weeks' gestation has an important contribution to the overall prevalence of spontaneous early preterm delivery.

The study in Chapter 9 demonstrated that in euploid pregnancies there is a weak inverse association between free free ß-human chorionic gonadotropin (ß-hCG) and TSH. In trisomy 21 pregnancies free ß-hCG was increased and TSH was decreased and in trisomy 18 pregnancies free ß-hCG was decreased and TSH was increased. The data provide further support to the hypothesis that hCG rather than TSH may be the primary thyrotropic factor in early pregnancy. The study also showed that although serum TSH is altered in pregnancies with fetal trisomies 21 and 18 this measurement does not improve the performance of screening for these aneuploidies provided by fetal nuchal translucency, free ß-hCG and pregnancy associated plasma protein-A.

The study in Chapter 10 established normal ranges of maternal thyroid function in twin pregnancies at 11-13 weeks' gestation. The study demonstrated that in normal twin pregnancies at 11-13 weeks' gestation, compared to singletons, maternal serum FT3 and FT4 are not significantly different but TSH is about 40% lower. The most likely explanation for the low TSH is the high level of free ß-hCG, which is twice as high as in singletons. The levels of serum TSH, FT3 and FT4 were similar in dichorionic and monochorionic twins, with or without twin-to-twin transfusion syndrome and there were no significant differences between the three groups in serum free ß-hCG.

11.2 SUGESTIONS FOR FUTURE STUDIES

The reference ranges established in this thesis can form the basis for routine screening for subclinical maternal thyroid dysfunction at 11-13 weeks' gestation. The controversy concerning the value of routine screening and treatment of subclinical hypothyroidism will ultimately be resolved through randomised studies demonstrating

that such policy would improve outcome both in terms of reducing pregnancy complications and adverse long term neurodevelopment in children. When such studies are undertaken it is likely that the gestational age at screening will be at 11-13 weeks because both in the UK and in many other countries women attend for such a visit as part of the well established routine screening for aneuploidies.

In women with hypothyroidism treated by thyroxine we found that although the level of serum FT4 was invariably normal or increased in a high proportion of cases there was high TSH and low FT3, high TSH and normal FT3 or normal TSH and low FT3. Consequently, if the objective in the treatment of hypothyroidism in pregnancy is to normalize the levels of the biologically active FT3 it is not useful to monitor the levels of FT4 but it is essential to measure the levels of both TSH and FT3. Recommendations on whether the objective in the treatment of hypothyroidism in pregnancy is to normalize TSH and / or FT3 rather than FT4 should ultimately be based on the results of major prospective studies examining the differential incidence of adverse pregnancy outcomes in the groups with low FT3 and normal TSH and FT4 and in those with high TSH and normal FT4 and FT3 compared to those in which all three biochemical markers are normal.

We found that previously undiagnosed hypothyroidism diagnosed at 11-13 weeks of gestation may be a contributing factor to about 5% of subsequent fetal losses. The extent to which the diagnosis of subclinical hypothyroidism and appropriate therapy can prevent fetal loss and the cost-effectiveness of such strategy require further investigation.

Our finding that the performance of first trimester prediction of PE by biophysical and biochemical markers can be improved by the addition of serum TSH will require confirmation by prospective studies. Similarly, the extent to which the prevalence of PE can be reduced by early screening for thyroid dysfunction and its appropriate therapy will require investigation by randomised studies.

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